Assessment of Serum Aldo-Keto Reductase Family 1 Member B10 for Early Diagnosis of Hepatocellular Carcinoma Patients in Suez Canal University Hospital, Egypt

Mariam Maher Makram Adeeb[1], Fikry Goubran Eskander[1], Fawzy Attia Khalil[2], Naglaa Elwy Salem[1]

[1]Department of Clinical and Chemical Pathology. Faculty of Medicine, Suez Canal University, Egypt.
[2]Department of Internal Medicine, Faculty of Medicine, Suez Canal University, Egypt.

Corresponding Author Mariam Maher

Mobile:
01224082871

E mail:
maruomamaher789@gmail.com.

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Background and study aim: Hepatocellular carcinoma is a challenging malignancy of global importance as it is considered the 2nd cause of mortality worldwide. This study aimed at finding additional blood biomarkers for hepatocellular carcinoma that may help in early diagnosis in Egyptian patients.

Methods: This comparative analytical study was conducted at the Clinical Pathology laboratory at Suez Canal University Hospital, Ismailia. A total sample of 88 participants were recruited by simple random sampling and divided into 4 equal groups, early and intermediate, late-stage HCC, cirrhotic patients without HCC and apparently healthy control. All study groups were subjected to an interview questionnaire and laboratory investigations like ALT, AST, PT, albumin, total bilirubin, INR, AFP and AKR1B10.

Results: The mean age was 63.6 ± 5.1 years 67.6 ± 5.9 years, 65.1 ± 4.1 years and 64.9 ± 7.0 years among four groups respectively. Males were higher among the HCC group. AFP showed significant differences between the late HCC and three other groups. AKR1B10 was significantly higher in early to moderate HCC than the other three groups. AKR1B10 could significantly detect early to moderate hepatocellular carcinoma with sensitivity of 72.7%, specificity of 89.4% at cut off value ≥ 0.91ng/ml. While AFP had a lower diagnostic value for detecting early to moderate hepatocellular carcinoma as it had sensitivity of 63.6%, specificity of 74.2% at cut off value ≥ 6.9 ng/ml.

Conclusion: AKR1B10 can help in early recognition of hepatocellular carcinoma in highly suspicious patients with good diagnostic value.

INTRODUCTION

Given that it is the 2nd cause that leading to mortality globally and the 6th most frequent cancer, hepatocellular carcinoma (HCC) is a difficult cancer of major global significance[1]. In Egypt, hepatocellular carcinoma is the 4th most prevalent cancer. Egypt is the third in Africa and the fifteenth worldwide as most populous country [2].

Chronic hepatitis B and C virus infections, non-alcoholic fatty liver disease, excessive use of alcohol, a family history of the disease, being obese, diabetes mellitus type 2, and smoking are all risk factors for hepatocellular carcinoma. Other risk factors include a family history of the disease [3].

The most common known risk factor for hepatocellular carcinoma is cirrhotic liver especially that associated with virus B and C infection [4]. The actual important factors to make the prognosis of HCC better, is early detection of disease, because the treatment, which may be surgical resection or interventional radiology or liver transplantation ,will be more effective and benefitable than in the advanced stage [5].

There are many blood biomarkers for early diagnosis of HCC which are classified into genetic biomarkers, clinical biomarkers and protein biomarkers. Genetic biomarkers like

circulating cell-free DNA, circular RNAs and microRNA, and clinical biomarkers like pre-albumin, D-Dimer, alanine transferase (ALT), activated partial thromboplastin time and mean platelet volume [6].

Protein biomarkers are classified into 3 groups: the traditional group, the emerging group and the autoantibody group. Unfortunately, none of the traditional groups are ideal in clinical diagnosis of HCC. But Aldo keto reductase family 1 member b10 (AKRF1MB10) ,which is one of the emerging groups, may show a promising diagnostic value in HCC [6,7]

Most people with early-stage HCC are asymptomatic, and liver function is unaffected. For surveillance, ultrasound (U/S) and serum alpha feto protein (AFP) level testing in blood are advised only for patients with high risk factors for HCC. The most often reported HCC biomarker worldwide is AFP [8].

As using U/S and AFP in diagnosis HCC is inaccurate because in early stage U/S could not determine benign from malignant nodules in the small cirrhotic liver , and not all patients are positive for AFP in this stage , so finding a promising biomarker is important for early diagnosis of HCC [9].

The Aldo-keto reductase superfamily includes the protein that is commonly referred to as AKR1B10. It is overexpressed in many other tumors besides HCC, such as breast cancer and cancer of lung, and it is expressed in the gastrointestinal tract. Other types of tumors, such as lung cancer, also express it [10].

As AKR1B10 may show a promising diagnostic value in Egypt, we estimated its level among HCC patients at Suez Canal University Hospital. So, this study aimed at Finding additional blood biomarkers for HCC that may help in early diagnosis in Egyptian patients with the main objectives To identify the role of serum (AKR1B10) among HCC patients and To estimate its sensitivity in early diagnosis of HCC.

PATIENTS AND METHODS

Study design: This was a comparative analytical cross-sectional study.

Study setting: Patients recruited from the hepatic outpatient clinic and oncology department of the Suez Canal University Hospital, Ismailia.

Study population: First group: early and intermediate stage HCC, second group: late-stage HCC, third group: cirrhotic liver patients without HCC. And fourth group: apparently healthy people.

Participants aged from 18 to 80 years for both gender. According to The Barcelona Clinic Liver Cancer (BCLC) Staging System, Group I includes very early, early, intermediate stage HCC. Group II includes advanced and terminal stage HCC. Regarding The Four-Stage Cirrhosis Classification System Group III includes patients with liver cirrhosis without HCC (including all stages of cirrhosis: compensated and decompensated). Group IV includes apparently healthy people. Participants who refused to participate were excluded from this study.

Sample size:

Using the following equation [11] with 95% confidence interval and 80% power:

$$n = 2\left[\frac{(Z_{\alpha/2} + Z_{\beta}) * \sigma}{\mu_1 - \mu_2}\right]^2$$

σ = the estimate of the standard deviation=2.4 ng/ml [7].

μ_1 = mean serum AKR1B10 level in HCC patients=3.27 ng/ml [7].

μ_2 = mean serum AKR1B10 level in chronic hepatitis patients =1.24 ng/ml. [7]

So, the sample size equals 22 subjects per group, the total sample size equals 88 subjects.

The participants enrolled in this study by simple random sampling.

Data collection:

All study groups were subjected to interview questionnaire including sociodemographic data, present history: onset, course, duration of symptoms and past history and drug history.

Laboratory Investigations were done to all participants as Several biochemical markers and liver enzymes, such as alanine aminotransferase (ALT), international normalized ratio (INR), aspartate aminotransferase (AST), albumin, alpha-fetoprotein (AFP), total bilirubin and prothrombin time, and were measured and evaluated. In addition, the AKR1B10

concentration in the serum was determined using sandwich ELISA and an AKR1B10 ELISA kit. [12]

Preparations:

Before beginning the test, ensure that all of the reagents, standard solutions, and samples have been brought to room temperature. Additionally, determine how many test strips will be required for the procedure. Insert the strips into the frames so that they can be used. Standard volume of 50 µl is being poured into the standard well. After adding 10 µl of anti-AKR1B10 antibody to the sample wells and 50 µl of streptavidin-HRP to the sample wells, pour 40 µl of the sample into each sample well.

Statistical analysis

The data that were gathered were input into a computer, and then the Statistical Package for Social Science SPSS version 26 program which was used to perform statistical analysis on them. Using the Shapiro Walk test, we checked to see if the data followed a normal distribution. The qualitative data were presented using frequencies and relative percentages as their respective representations. To determine the degree of dissimilarity between the qualitative variables under consideration, a Chi-square test (2) was carried out. Calculating the difference between quantitative variable in ≥ 2 groups was accomplished with the help of a Kruskal Wallis test and one-way ANOVA, respectively for parametric and non-parametric variables. The relationship between two parametric and non-parametric quantitative variables was analyzed with the use of the Spearman correlation and Pearson correlation tests, respectively. ROC curve for the detection of validity in contrast to the cut-off point used to ensure diagnostic accuracy. If p value was < 0.05, it was considered significant.

RESULTS

A total sample of a total of 88 participants were classified into 4 equal groups: First group: early and intermediate stage HCC. Second group: late-stage HCC. Third group: cirrhotic liver without HCC. Fourth group: apparently healthy people. The mean age was 63.6± 5.1 years, 67.6± 5.9 years, 65.1± 4.1years and 64.9± 7.0 years among groups I, II, III and IV respectively, with no significant difference. The gender was comparable between the four groups: 81.8%, 72.7%, 77.3% and 54.5% males among the four groups respectively **(Table 1).**

According to our results, PT showed significant differences between the participating groups. INR was significantly higher among early and late HCC and liver cirrhosis groups than in control groups. Total bilirubin was significantly the highest among the late HCC group rather than the early HCC, cirrhotic and control groups. Albumin significantly differed between groups, but there was no significant difference between the early HCC group and the control group. AST was higher among early to moderate HCC, severe HCC and cirrhotic liver groups than control. Also, significant difference was reported between the early and late groups regarding AST. ALT was higher among early to moderate HCC, severe HCC and cirrhotic liver groups than control **(Table 2).**

AKR could significantly detect early to moderate hepatocellular carcinoma with sensitivity of 72.7%, specificity of 89.4% and area under the curve of 0.874 at cut off value ≥ 0.91. While AFP had a lower diagnostic value for detecting early to moderate hepatocellular carcinoma as it had sensitivity of 63.6%, specificity of 74.2% and area under the curve of 0.680 at cut off value ≥6.9 **(Table 3, Figure 1).**

AFP could significantly detect severe hepatocellular carcinoma with sensitivity of 68.2%, specificity of 69.7% and area under the curve of 0.794 at cut off value ≥ 4.35. While AKR had no statistical significant diagnostic value for severe hepatocellular carcinoma **(Table 4, Figure 2).**

AFP showed significant differences between severe HCC and three other groups; early, chronic liver disease and healthy groups. It was the highest level among HCC patients with a mild increase among liver cirrhosis patients. While AKR was significantly higher among groups with early to moderate HCC than the other three groups, severe HCC, chronic liver disease and control groups. Also, AKR was higher in the cirrhosis group than in controls **(Table 5).**

There was a weak (+) correlation between AKR and AFP (p=0.001). While AFP had a positive moderate correlation with PT, INR, total bilirubin, AST and ALT and a weak negative correlation with albumin. There was no

significant correlation with other parameters. Regarding AKR, it had a significantly weak positive correlation with AST and ALT and no other significant correlation with other parameters **(Table 6, Figure 3).**

Table 1. Basic characteristics of the participated groups.

Variable		Early HCC n= 22	Late HCC n= 22	Cirrhosis without HCC n= 22	Control n=22	P value
Age (years)	Mean ± SD	63.6± 5.1	67.6± 5.9	65.1±4.1	64.9± 7.0	0.136[a]
Gender	Female, n (%)	4 (18.2)	6 (27.3)	5 (22.7)	10 (45.5)	0.234[b]
	Male, n (%)	18 (81.8)	16 (72.7)	17 (77.3)	12 (54.5)	

*a; Kruskal Wallis test, b; Chi square test; *p is significant at <0.05; HCC; Hepatocellular Carcinoma*

Table 2. Comparing laboratory data between the participated groups.

Variable	Early HCC Mean ± SD	Late HCC Mean ± SD	Cirrhosis without HCC Mean ± SD	Control Mean ± SD	P value
PT (seconds)	15.5±3.6	17.4± 4.0	14.6± 2.5	13.0± 0	**<0.001*abef**
INR	1.2± 0.2	1.4± 0.3	1.2± 0.2	1± 0	**<0.001*abcef**
Total bilirubin (mg/dl)	1.2± 0.9	6.5± 9.3	1.08 ± 0.8	0.7± 0.2	**<0.001*abf**
Albumin (g/dl)	3.6± 1.0	2.6± 0.5	3.1± 0.7	3.7± 0.5	**<0.001*abcdf**
AST (IU/L)	37.3± 25.1	77.6± 58.6	45.4± 50.9	16.7± 1.9	**<0.001*abcf**
ALT (IU/L)	27.2± 14.6	48.2± 55.1	41.2± 95.0	16.4± 3.3	**0.001*e**

*One way ANOVA; *p is significant at <0.05*
Post hoc test (LSD) was used to assess difference between each 2 groups
a; significance between group I and II
b; significance between group II and III
c; significance between group III and IV
d; significance between group I and III
e; significance between group I and IV
f; significance between group II and IV
HCC; Hepatocellular Carcinoma, PT; Prothrombin Time, INR; international normalized ratio, AST; aspartate aminotransferase, ALT; Alanine aminotransferase

Table 3. Diagnostic value of AKR and AFP in diagnosis of early to moderate hepatocellular carcinoma.

Variable	AUC	Cut off value	Sensitivity	Specificity	P value
AKR (ng/ml)	0.874	0.91	72.7%	89.4%	**<0.001***
AFP (ng/ml)	0.680	6.9	63.6%	74.2%	**0.008***

AUC; area under the curve, AKR; Aldo-Keto Reductase, AFP; alpha-fetoprotein
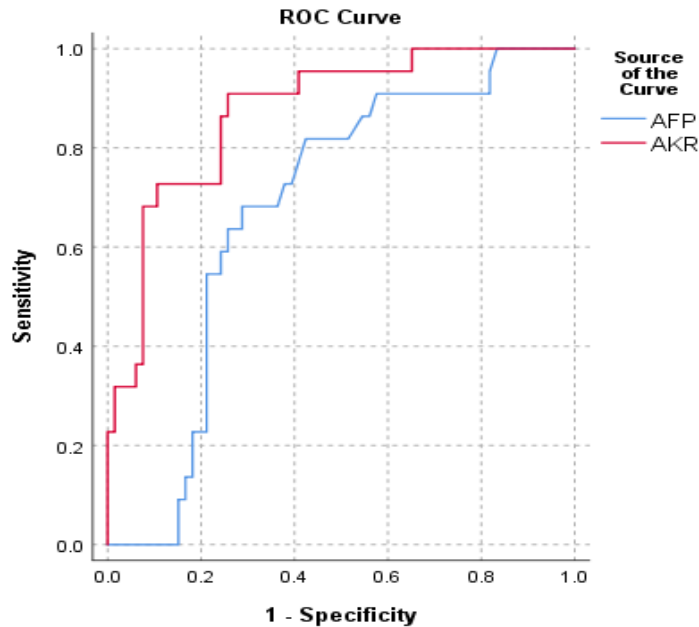
ROC Curve

Figure 1:
Legend: ROC curve analysis of AKR and AFP in diagnosis of early to moderate hepatocellular carcinoma.
Caption: AKR could significantly detect early to moderate hepatocellular carcinoma with sensitivity of 72.7%, specificity of 89.4% and area under the curve of 0.874 at cut off value $\geq$ 0.91.

Table 4. Diagnostic value of AKR and AFP in diagnosis of severe hepatocellular carcinoma.

Variable	AUC	Cut off value	Sensitivity	Specificity	P value
AKR (ng/ml)	0.395	0.61	40.9%	45.5%	0.092
AFP (ng/ml)	0.794	4.35	68.2%	69.7%	**<0.001***

AUC; area under curve, AKR; Aldo-Keto Reductase, AFP; alpha-fetoprotein
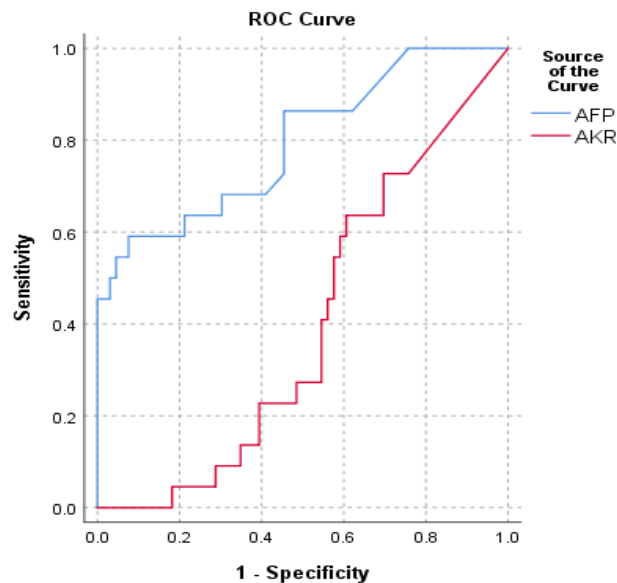
ROC Curve

Figure 2:
Legend: ROC curve analysis of AKR and AFP in diagnosis of severe hepatocellular carcinoma.
Caption: AFP could significantly detect severe hepatocellular carcinoma with sensitivity of 68.2%, specificity of 69.7% and area under the curve of 0.794 at cut off value $\geq$ 4.35.

Table 5. Comparing AFP and AKR between the four participated groups.

Variable	Early HCC Mean ± SD	Late HCC Mean ± SD	Cirrhosis without HCC Mean ± SD	Control Mean ± SD	P value F test
AFP (ng/ml)	135.6± 232.4	22978.2± 58232.2	9.4± 26.4	1.3± 0.9	**<0.001***
P value (post hoc test)	a= **0.011*** b= **0.011*** c= 0.999			d= 0.989 e= 0.988 f= **0.011***	
AKR (ng/ml)	1.2± 0.5	0.6± 0.2	0.8± 0.4	0.4± 0.4	**<0.001***
P value (post hoc test)	a= **<0.001*** b= 0.050 c= **0.001***			d= **<0.001*** e= **<0.001*** f= 0.129	

*Kruskal Wallis test; *p is significant at <0.05*
Post hoc test (LSD) was used to assess difference between each 2 groups
a; significance between group I and II
b; significance between group II and III
c; significance between group III and IV
d; significance between group I and III
e; significance between group I and IV
*f; significance between group II and IV*
AKR; Aldo-Keto Reductase, AFP; alpha-fetoprotein

Table 6. Correlation between AKR, AFP and other laboratory data.

Variable	AKR		AFP	
	r	p	r	p
Age (years)	-0.096	0.374	-0.002	0.986
PT (seconds)	0.066	0.541	0.351	**<0.001***
INR	0.130	0.228	0.409	**<0.001***
Total bilirubin (mg/dl)	-0.001	0.994	0.459	**<0.001***
Albumin (g/dl)	0.045	0.679	-0.297	**0.005***
AST (IU/L)	0.259	**0.015***	0.518	**<0.001***
ALT (IU/L)	0.229	**0.032***	0.511	**<0.001***
AFP (ng/ml)	0.344	**0.001***	-	-

*spearman correlation, *p is significant at <0.05*
HCC; Hepatocellular Carcinoma, PT; Prothrombin Time, INR; international normalized ratio, AST; aspartate aminotransferase, ALT; Alanine aminotransferase, AKR; Aldo-Keto Reductase, AFP; alpha-fetoprotein
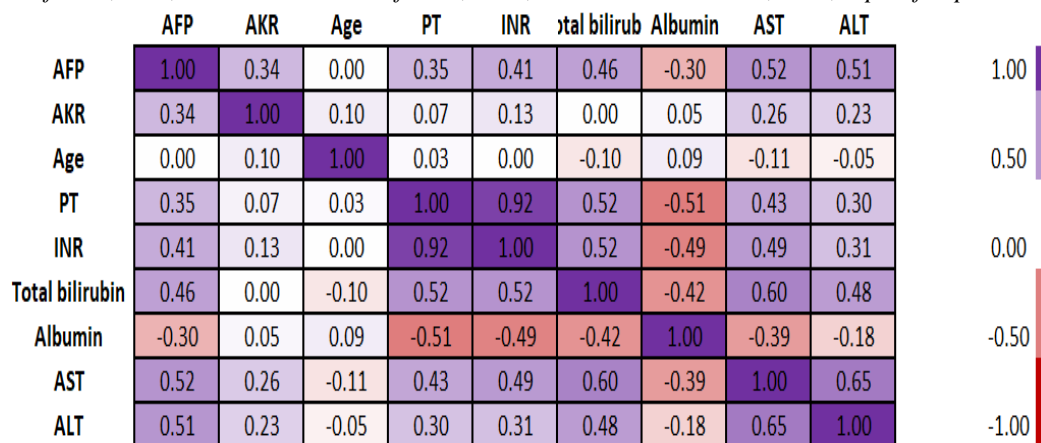
Figure 3:
Legend: Correlation matrix showing the strength of association between AFP, AKR and other parameters.
Caption: There was a weak positive correlation between AKR and AFP (p=0.001). While AFP had a positive moderate correlation with PT, INR, total bilirubin, AST and ALT and a weak negative correlation with albumin. There was no significant correlation with other parameters. Regarding AKR, it had a significantly weak positive correlation with AST and ALT and no other significant correlation with other parameters.

DISCUSSION

This comparative analytical study was conducted with the aim of finding additional serum biomarker for HCC that may help in early diagnosis in Egyptian patients.

A total sample of 88 participants were enrolled in this study and classified into 4 equal groups for first group: early and intermediate stage HCC, second group: late-stage HCC, third group: patients with liver cirrhosis without HCC and fourth group: apparently healthy people. The current study found that the age was, 63.6± 5.1 years among early to moderate HCC group, 67.6± 5.9 years in the late group, 65.1± 4.1 years in the cirrhotic group and 64.9± 7.0 years in the control group, with no difference. Gender was comparable between the four groups: 81.8%, 72.7% ,77.3% and 54.5% males among the four groups respectively. Males were higher among the HCC group with no significant difference between groups.

A similar study by **Han [7]** had three levels of liver diseases and a healthy control group. The mean age among the HCC group was 57 (33-78) years, 55 (31-75) years among the liver cirrhosis group, 36 (23-57) years among the chronic hepatitis group and 32 (28-40) years among healthy controls. Males were higher among the HCC group but not significantly assessed **Han [7]**. This goes in line with **Matkowskyj [13]** who found similar age ranges among HCC patients with predominance of male gender than females.

In Egypt, **Gadallah [14]** compared HCC patients, liver cirrhosis patients and controls with mean age of 59.4 ± 6.00, 57.9 ± 5.04 and 55.8 ± 11.1 years respectively with no significant difference. Males reported a higher percentage than females among HCC patients. These results go in line with **Sato [15]** regarding comparable age for HCC and healthy controls.

This study reported that INR was significantly higher among early and late HCC and liver cirrhosis groups than in control groups. This was in line with **Han [7]** as INR was higher among HCC group and cirrhotic group than the control group.

In agreement with this study **Liang [16]** found that HCC patients had high INR compared to cirrhotic patients without HCC with significant difference (p= 0.026). Similarly, **Tahon [17]** revealed that INR was significantly differ between HCC, cirrhosis and healthy control groups (1.61±0.21, 1.52±0.49, vs 1.03±0.05; p less than 0.001).

In disagreement with our results, **Gadallah [14]** found no significant difference in INR among HCC, liver cirrhosis and healthy control groups.

Regarding our results, total bilirubin was significantly the highest among the late HCC group rather than in the early HCC, cirrhotic and control groups.

Similarly **Han [7]** found that HCC had highest level of total bilirubin than liver cirrhosis and control groups (59.19±90.31 vs 47.23±74.16 and 11.50±3.96 µmol/l). In agreement, **Gadallah [14]** admitted that total bilirubin was significantly higher among HCC compared to liver cirrhosis patients and healthy control groups.

Based on our study findings, healthy controls had significantly higher serum albumin levels than early to moderate and late HCC and liver cirrhosis groups. Additionally **, Han [7]** demonstrated the same results as healthy controls had higher levels of serum albumin than HCC patients and liver cirrhosis patients (39.81±3.75 vs 32.82±6.03 and 33.52±6.79 g/L).

The same results were reported by **Gadallah [14],** serum albumin was higher among healthy controls than in HCC and liver cirrhosis patients with significant differences.

According to the findings of this study, ALT was higher among early to moderate HCC than among control with significant differences. Like our results, **Han [7]** reported significant difference in the level of ALT between HCC and liver cirrhosis groups compared to healthy controls.

In accordance, **Kobeisy [18]** found a high ALT was associated with HCC in late stages (95% CI 8.27 (3.47–19.70); p <0.001). In agreement **, Tahon [17]** found that ALT was higher among HCC patients than cirrhotic patients and controls with significant difference (80.53U/L±26.96, 41.00U/L±37.46, vs 23.47U/L±3.83; p <0.001).

Gadallah [14] agreed that ALT was higher among HCC patients and liver cirrhosis patients than healthy controls. And **Hsu [19]** agreed that ALT was higher among HCC patients than healthy controls. In consistence with our results, **Sato [15]** revealed higher ALT levels among HCC patients than healthy controls.

In contrast, **Mori [20]** found that ALT was higher among group without HCC in comparison with group with HCC but with no significant difference (89 U/L vs 65 U/L respectively).

In this study, AST was higher among early to moderate HCC, late HCC and cirrhotic liver groups than control. Similar to our results**, Han [7]** reported significant difference in the level of AST between the HCC group and the liver cirrhosis group when compared to healthy controls. In addition to that, **Gadallah [14]** found higher levels of AST among HCC patients and liver cirrhosis patients than controls. Similar results were reported by **Hsu [19]** who agreed that AST level was higher among HCC patients than controls.

In agreement**, Tahon [17]** found that AST was significantly higher among HCC patients than cirrhotic patients and controls with significant difference ($103.63U/L\pm32.18$, $53.37U/L\pm30.55$, vs $23.80U/L\pm2.40$; $p < 0.001$). Additionally**, Kobeisy [18]** found that high AST was significantly associated with late stages of HCC (95% CI 4.24 (1.94–9.25); $p < 0.001$) and **Sato [15]** also found that AST showed higher levels among HCC positive group compared with HCC negative group (97U/L vs 43U/L respectively; $p < 0.001$).

In the current study, AFP showed significant differences between late HCC and three other groups: early, cirrhotic and control groups. It was the highest level among HCC patients with a mild increase among liver cirrhosis patients.

Also, **Han [7]** demonstrated that AFP showed higher levels in the late stages of HCC than the early to intermediate stages of HCC.

In consistency, another Egyptian study by **Kobeisy [18]** found that high AFP (AFP >10 ng/ml) was significantly associated with HCC and fibrosis especially late stages III/ IV. **Bruden [22]** reported that AFP levels has been used to screen high risk people for human hepatocellular carcinoma, including those with cirrhosis or HCV.

The results of a screening for HCC who were chronically infected with hepatitis B are shown here.by **McMahon [23]**, they agreed that AFP was effective in detecting most HCC tumors. This was accepted also in a systematic review by **Gebo [24]** who agreed that HCC was detected in patients who had screening two times/ year with serum alpha-fetoprotein.

In agreement, **Ye [25]** reported increased levels of serum AFP among HCC patients and to some extent liver cirrhosis patients. Similar to our results, **Gadallah [14]** found that AFP and HCC were associated. As it showed higher levels among HCC than liver cirrhosis and controls.

Based on **Hsu [19]** results, AFP showed significantly higher levels associated with hepatocellular carcinoma. This goes in line with another study by **Sato [15].**

Considering our suggested biomarker, AKR1B10 was significantly higher among groups with early to moderate HCC than three other groups: late HCC, liver cirrhosis and control groups. Which in turn indicates that AKR1B10 ($p<0.001$) had a close relation with early development of hepatocellular carcinoma.

Additionally, **Ye [25]** reported that patients with HCC had much greater levels of AKR1B10 than the control group did, and this difference was statistically significant (p 0.001). Also, AKR1B10 revealed a slight rise among patients with liver cirrhosis. A study by **Zhu [26]** agreed that AKR1B10 was significantly associated with early diagnosis of HCC and associated with the risk of HCC ($p< 0.05$). **Jin [27]** reported that significant upregulation of AKR1B10 was observed in tandem with the incremental development of hepatocarcinogenesis (from liver cirrhosis to moderately differentiated HCC) ($p <0.001$).

Tsuzura [28] stated that compared to normal livers, livers with chronic hepatitis or cirrhosis preneoplastic diseases that underlie HCC—had significantly greater levels of AKR1B10, and AKR1B10 expression was still higher in HCC ($p < 0.001$). To add to that, **Han [7]** agreed that AKR1B10 level showed significantly ($p< 005$) higher levels among early and intermediate stages of HCC than the late and terminal stages of HCC. On the same hand, **Matkowskyj [13]** described a significant association between AKR1B10 levels and hepatocellular carcinoma. As AKR1B10 level was higher among HCC patients when compared with liver cirrhosis and controls ($p <0.05$).

AKR1B10 was found with a significant ($p<0.001$) high level among HCC patients in a study by **Gadallah [14].** This goes in line with a study compared between animals and humans with HCC by **Torres-Mena [29]** who suggested

that AKR1B10 level was increased in HCC and could be used as a biomarker of it (p <0.05).

This study found that there was a weak positive correlation between AKR1B10 and AFP (p=0.014). This goes in line with **Ye [25]** who found an association between AKR1B10 and AFP (p <0.05). Similarly, **Zhu [26]** found that AKR1B10 was significantly associated with AFP. This agrees with the study by **Gadallah [14]** who found a correlation between AKR1B10 and AFP.

Our current results found that AKR1B10 had no relation with ALT and AST. Similarly, **Gadallah [14]** found no correlation between AKR1B10 and ALT but correlation with AST was found significant. In the same line, **Mori [20]** agreed that ALT levels were not correlated with AKR1B10 expression.

Unlike our results, **Ye [25]** reported an association between AKR1B10 and ALT and AST.

Based on the results of this study, AKR1B10 could significantly detect early to moderate hepatocellular carcinoma with sensitivity of 72.7%, specificity of 89.4% and area under the curve of 0.874 at cut off value ≥ 0.91ng/ml. While AFP had a lower diagnostic value for detecting early to moderate hepatocellular carcinoma as it had sensitivity of 63.6%, specificity of 74.2% and area under the curve of 0.680 at cut off value ≥6.9ng/ml.

AFP could significantly detect late stage of hepatocellular carcinoma with sensitivity of 68.2%, specificity of 69.7% and area under the curve of 0.794 at cut off value ≥ 4.35 ng/ml. While AKR had no statistically significant diagnostic value to severe hepatocellular carcinoma.

Bruce [30] reported that an elevated AFP with cut off > 8 ng/ml has a high positive predictive value (78%) for HCC with 39% sensitivity and 95% specificity. Additionally, **Zhu [26]** revealed that the the area under the curve (AUC) for AKR1B10 in serum was 0.866 with a 95 percent confidence interval (CI) of 0.826---0.907, and the best diagnostic cut-off was 232.7 pg/mL.. **Ye and colleagues [25]** agreed that the optimal diagnostic cut off value of AKR1B10 level was 267.9 pg/ml, which had a sensitivity of 64.4%, specificity of 92.3% and an AUC of 0.831. on the other hand, AFP showed at cut off value of 20.2 ng/ml a sensitivity of 59.5% and a specificity of 77.7%.

Similar to our results, **Kanno [31]** studied different stages of liver cirrhosis and reported that serum AKR1B10 at a cut off value of 1.03 ng/ml can predict late stage of cirrhosis with early HCC detection. Its sensitivity was 71.4% and specificity of 94.7%.

According to the Egyptian study by **Gadallah [14]**, AKR1B10 had a good diagnostic value of early to moderate HCC at a cut off value of 0.945 ng/ml, reported sensitivity of 86.7% and specificity of 70%. While AFP showed at cut off 17.9 ng/ml, a sensitivity of 67% and a higher specificity of 88%. Which goes in line with our results that AKR1B10 had a higher diagnostic value for detection of early HCC than AFP.

Similar to that **Mori [20]** who agreed that due to the association between high hepatic AKR1B10 expression and an increased risk of developing HCC, AKR1B10 can predict the incidence of HCC. To add to that, **Sato [21]** demonstrated that the adjusted hazard ratio (HR) showed that high AKR1B10 expression was a significant independent predictor of HCC formation and that AKR1B10 expression 6% was connected to a more than sixfold increased risk of HCC development.

The difference in the diagnostic value of both AKR1B10 and AFP may suggest that AKR1B10 plays a role in the pathogenesis of hepatocellular carcinoma and developed earlier than AFP **[32]**.

CONCLUSION

AKR1B10 has better diagnostic value than AFP in detection of early stages of HCC as it had good sensitivity (72.7 %) and higher specificity (89.4%). And it has higher levels in the early to moderate stages of HCC (1.2± 0.5 ng/ml) than in the severe late stages (0.6± 0.2 ng/ml). So AKR1B10 can help in early detection of HCC in highly suspicious patients with good diagnostic value.

This study recommended that AKR1B10 can be used as a regular screening laboratory investigation among preneoplastic patients. We also recommend that AKR1B10 together with ultrasonography should be performed every six months for those patients with high-risk of HCC. Further studies with bigger sample size and in other areas of the country should be done to

investigate the role of AKR1B10 in the pathogenesis of hepatocellular carcinoma and to generalize our results.

Ethical considerations

Clearance for ethical standards was acquired from the ethics committee of the Faculty of Medicine at Suez Canal University. Every participant provided their written informed permission, which was collected.

HIGHLIGHTS

- AKR1B10 could significantly detect early to moderate hepatocellular carcinoma with sensitivity of 72.7%, specificity of 89.4% at cut off value ≥ 0.91ng/ml .

- AKR1B10 can help in early recognition of hepatocellular carcinoma in highly suspicious patients with good diagnostic value.

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