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The Value of Serum MidKine as a Marker of Hepatocellular Carcinoma on Top of Hepatitis C Virus Related Cirrhosis

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Abstract

Background: Hepatocellular carcinoma (HCC) is characterized by the deficiency of specific symptoms in the early stages of the disease and a poor prognosis. Biomarkers that have the diagnostic ability to distinguish between inflammation, cirrhosis, and HCC are needed for early case detection. MidKine (MK) is overexpressed in many malignancies, including hepatocellular carcinoma, gastric carcinoma, colon carcinoma, lung carcinoma, urinary bladder carcinoma and prostate carcinoma. **Objective:** The objective is to evaluate serum MidKine as a marker of HCC in HCV-related liver cirrhosis. **Patients and Methods:** This study included 50 patients with HCC (on top of hepatitis C virus-related cirrhosis), 20 cirrhotic patients due to chronic HCV infection, and 10 normal subjects as a control group. History, examination, and laboratory investigations were performed on all subjects in the three groups including MK and Alpha fetoprotein (AFP) serum levels. **Results:** Serum levels of AFP and MK were higher in the HCC group than in the other study groups ($P < 0.001$). AFP was positive in 35 (70%) and negative in 15 (30%) whereas MK was positive in all HCC cases. There was a statistically significant correlation between MK serum levels and AFP serum levels, tumor size, and tumor stage. **Conclusion:** MidKine is a novel sensitive biomarker that could be used for early detection of HCC and has a high significance in differentiation between cirrhosis and HCC cases. MidKine levels are correlated with HCC stages and sizes.

Keywords

MidKine, Hepatocellular Carcinoma, Alpha-Fetoprotein, Chronic Hepatitis C

1. Introduction

Hepatocellular carcinoma (HCC) is the most common primary malignant disease of the liver. In developed countries; it is the third leading cause of cancer-related deaths. It is the sixth most common cancer; in Western countries, more than one million people die from HCC each year [1].

Most HCC cases (approximately 80%) are associated with cirrhosis due to chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infection. In particular, cirrhotic patients with HCV infection have the highest 5-year cumulative risk of developing HCC [2]. In Egypt, HCC is one of the most common malignancies and a leading cause of death due to the high prevalence of cirrhosis related to chronic HCV [3].

The options for curative treatment of HCC are usually very limited at diagnosis because tumor has often reached an advanced stage. Therefore, early diagnosis is necessary to cure the patient and avoid increasing healthcare costs [4].

Liver disease progression into liver cancer is primarily monitored by serum levels of the oncofetal glycoprotein, alpha-fetoprotein (AFP), or the core fucosylated glycoform of AFP (AFP-L3) [5].

AFP is not positive in all patients with HCC and it can be produced in a variety of circumstances, including other liver diseases. Therefore, more sensitive and specific serum biomarkers for HCC are desired and the use of AFP in HCC screening is not fully accepted [6].

MidKine (MK) was originally discovered in embryonic carcinoma cells and is involved in the early stage of retinoic acid-induced differentiation [7]. Studies have identified MK as an HCC serum marker and it has been identified as one of the five important potential novel biomarkers for early detection of HCC [8].

This study was conducted with the aim of evaluating serum MK as a marker of hepatocellular carcinoma in HCV-related liver cirrhosis.

2. Patients and Methods

2.1. Type of Study

This is a case-control study conducted at Mansoura Specialized Medical Hospital and Mansoura University Hospital.

2.2. Patients

The study included patients with HCC (on top of hepatitis C virus-related cirrhosis) (50 cases), cirrhotic patients due to chronic hepatitis C virus infection without confirmed HCC (20 cases), and normal subjects as controls (10 subjects).

We included patients of both sexes with the following criteria; age > 18 years, HCV positive patients by HCV antibody ELISA, and cirrhotic patients diagnosed by clinical, biochemical, and abdominal ultra-sonographic findings. Patients with the following conditions were excluded; co-infection with HBV or HIV, previous liver transplantation, malignancies other than HCC, and patients

with co-morbidity as advanced cardiac, respiratory, renal, neurologic, and rheumatologic disorders.

2.3. Methodology

In all cases, a complete history was obtained and a comprehensive general (with emphasis on signs of liver failure) and abdominal examination was performed. Laboratory investigations were performed on all the subjects of the three groups including liver function tests (serum albumin, serum bilirubin, prothrombin time (INR), ALT & AST), anti-HCV and HCV PCR, serum creatinine, and serum levels of Alpha-fetoprotein.

Determination of MK: the determination was performed using the Human MidKine ELISA Kit from the company (Shanghai Sunred Biological Technology Co., Ltd.—ELISA kit, China) (Cat. No. 201-12-1634).

Test principle of serum MidKine measurement using human MidKine enzyme-linked immunosorbent assay kit: MidKine was added to monoclonal antibody Enzyme well which was pre-coated with Human MidKine monoclonal antibody then, MidKine antibodies labeled with biotin were added to streptavidin-HRP to form immune complex. The wells were then incubated and washed to remove the uncombined enzyme. Chromogen Solution A, B were then added where the color of the liquid changes into blue, and at the effect of acid, the color finally becomes yellow. The chroma of color and the concentration of the human Substance MidKine of the sample were positively correlated. Using the standard density as the horizontal, the optical density (OD) value for the vertical draw the standard curve on graph paper, the corresponding density according to the sample OD value by the Sample curve (the result is the sample density) was detected and used to calculate the sample density result.

Abdominal ultrasound was performed in all cases and triphasic abdominal CT was performed for assessment of the tumor criteria in HCC cases group regarding size, number, and degree of infiltration of lesions.

3. Statistical Analysis

The collected data were coded, processed, and analyzed using the Statistical Package for Social Sciences (SPSS) version 22 for Windows® (IBM, SPSS Inc, Chicago, IL, USA). Qualitative data were presented as number (frequency) and Percent. Comparison between groups was done by Chi-Square test (χ^2) (or Fisher's exact test). Quantitative data were tested for normality by Kolmogorov-Smirnov test. Normally distributed data were presented as mean ± SD. One way ANOVA was used to compare between more than two groups (expressed as F). Non-parametric data were presented as median (min-max). Kruskal Wallis test (expressed as KW) was used for comparison between groups. Correlation of numeric data was done by Pearson's or Spearman correlation (r). The optimal cutoff value was serum MidKine level to differentiate between different groups was determined using Youden index J which is the farthest point on receiver op-

erator characteristic (ROC) curve and expressed in terms of sensitivity and specificity. For all tests, P values < 0.05 are considered significant.

4. Results

The mean age of cases showed no statistically significant difference between the study groups (P = 0.162) with the highest mean age in HCC group (52.15 years). Sex distribution showed a statistically significant difference between study groups as the majority of cases in the HCC group were male (70%) while the majority of cases in the control and cirrhosis groups were female (P = 0.002). All parameters tested showed a high level of significance between the different study groups, with the exception of hemoglobin (HGB) and white blood cells (WBCs) (P value = 0.119 and 0.331 respectively). In the HCC group, there were 5 Barcelona Clinic Liver Cancer (BCLC) stage 0 cases, 20 stage A cases, and 25 stage B cases. Regarding tumor size, 21 cases had tumor size < 3 cm, and 29 cases had tumor size ≥ 3 cm. There were variant numbers of tumors among the cases; 15 cases with one lesion, 20 cases with 2 lesions, 10 cases with 3 lesions, and 5 cases with ≥4 lesions (Table 1).

The median level of AFP in the HCC group was 110 IU/mL which was higher than the median value in the other study groups with high significance level between study groups (P < 0.001). The median level of MK in the control group

Table 1. Demographic data and laboratory investigations within the study groups.

Variable		Control group N = 10	Cirrhosis group N = 20	HCC group N = 50	P value
Age (Years)		50.40 ± 11.6	49.70 ± 6.8	52.15 ± 7.9	0.162
Gender	Males	4 (40%)	6 (30%)	35 (70%)	0.002*
	Females	6 (60%)	14 (70%)	15 (30%)	
HGB (gm/dL)		12.94 ± 1.81	11.52 ± 2.4	12.37 ± 2.4	0.119
PLTs (10^3/mm^3)		258.5 (176 - 324)	74.5 (22 - 136)	134 (80 - 312)	<0.001*
WBCs (10^3/mm^3)		6.05 (4.6 - 10.3)	6.26 (2.6 - 18.6)	6.2 (2.6 - 10.3)	0.331
Creatinine (mg/dL)		0.86 (0.51 - 1.1)	1.38 (0.35 - 2.7)	0.85 (0.45 - 1.4)	<0.001*
INR		1.06 ± 0.11	1.78 ± 0.36	1.23 ± 0.20	<0.001*
Albumin (gm/dL)		4.46 ± 0.27	2.79 ± 0.63	3.51 ± 0.69	<0.001*
Total bilirubin (mg/dL)		0.73 (0.50 - 1.1)	4.39 (0.30 - 20.6)	1.09 (0.39 - 2.11)	<0.001*
Direct bilirubin (mg/dL)		0.21 (0.19 - 0.38)	1.8 (0.12 - 12)	0.56 (0.15 - .9)	<0.001*
ALT (U/L)		33 (19 - 40)	43.5 (10 - 70)	46.5 (16 - 210)	0.010*
AST (U/L)		34 (22 - 40)	57.5 (10 - 97)	57 (28 - 320)	0.001*

HGB: hemoglobin. PLTs: platelets. WBCs: White blood cells, INR: international normalized ratio, ALT: alanine transferase, AST: aspartate transaminase, HCC: hepatocellular carcinoma.

was 3018 pg/mL, the median level in the cirrhosis group was 5165 pg/mL and the median level in the HCC group was 18,298 pg/mL with a high level of significance between the different study groups ($P < 0.0001$) (Table 2).

Among the HCC cases in this study, AFP was positive in 35 cases (70%) and negative in 15 cases (30%) whereas MK was positive in all HCC cases (Table 3).

There was a statistically significant correlation between MDK levels and AFP serum levels, tumor size, and tumor stage (Table 4).

Table 2. Comparison of the serum levels of AFP and MidKine in the study groups.

Variable	Control group N = 10	Cirrhosis group N = 20	HCC group N = 50	P value
AFP (IU/mL)	3.5 (1.2 - 27.2)[a]	26.5 (1.1 - 63)[b]	110 (2.6 - 1800)[a,b]	<0.001*
MidKine (pg/mL)	3018 (1296 - 5062)[a]	5165 (2747 - 7204)[b]	18,298 (9952 - 62,466)[a,b]	<0.001*

AFP: alpha-fetoprotein, HCC: hepatocellular carcinoma.

Table 3. Correlation between AFP and MK in detection of HCC cases.

HCC cases (50)		AFP in HCC cases	AFP in HCC cases
		Positive	Positive
MK in HCC cases	Positive	35	35
	Negative	0	0

MK: MidKine, HCC; hepatocellular carcinoma.

Table 4. Correlation between MidKine level and other parameters in the study.

	MidKine level	
	r	P
Age	−0.016	0.882
HGB	−0.101	0.349
PLTs	0.166	0.310
WBCs	0.123	0.429
Creatinine	0.091	0.784
INR	0.192	0.108
Albumin	−0.144	0.165
Bilirubin	0.160	0.133
ALT	0.160	0.327
AST	0.097	0.736
AFP	0.460	<0.001*
Tumor stage	0.452	<0.001*
Tumor size	0.517	<0.001*

HGB: hemoglobin; PLTs: platelets; WBCs: White blood cells; INR: international normalized ratio; ALT: alanine transferase; AST: aspartate transaminase; AFP: alpha-fetoprotein.

The cutoff point of MidKine to differentiate between the control group and the cirrhosis group was 3980 pg/mL with 84% sensitivity, 81% specificity, and total accuracy of 86%. The cutoff point of MidKine to differentiate between the cirrhosis group and the HCC group was 9020 pg/mL with a sensitivity of 96%, a specificity of 92%, and an overall accuracy of 97% (Table 5, Figure 1, Figure 2).

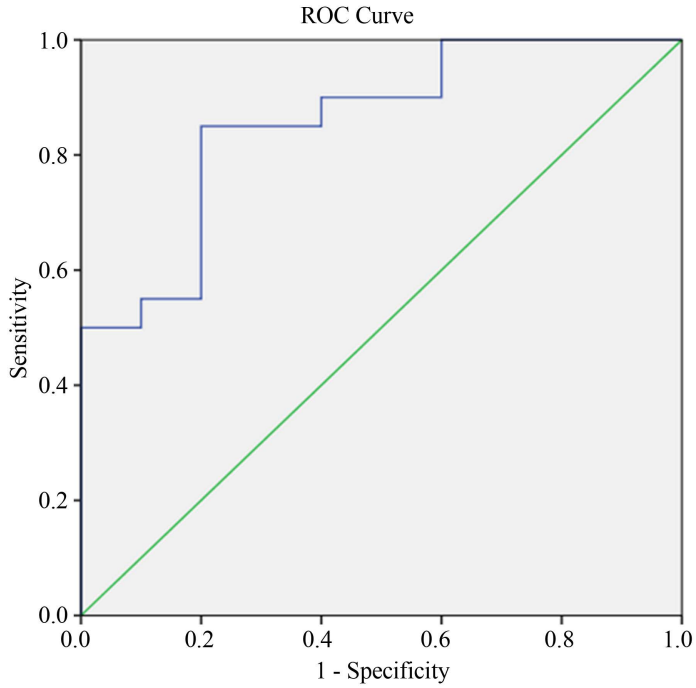

Figure 1. MidKine to differentiate between (control group & cirrhosis cases).

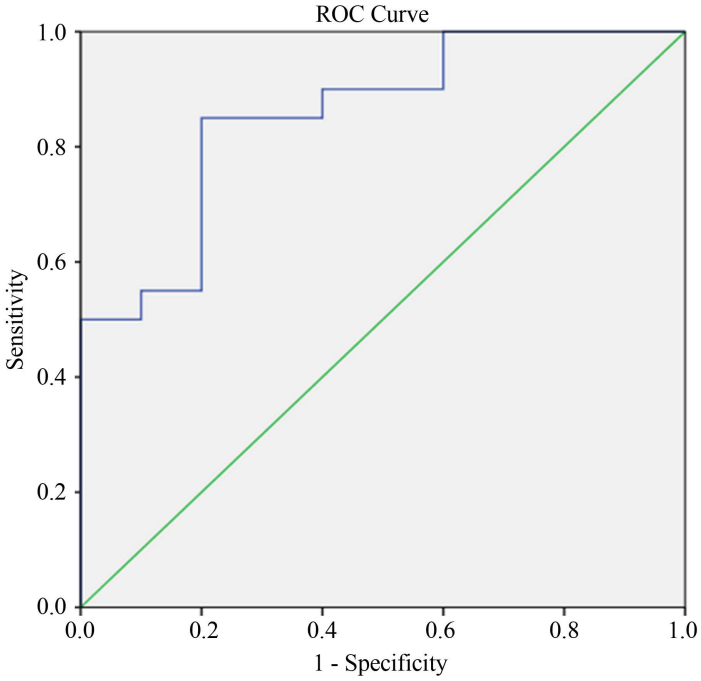

Figure 2. MidKine to differentiate between (control group & cirrhosis cases).

Table 5. Prediction of ability of MidKine to differentiate between (control group & cirrhosis cases) and (cirrhosis cases and HCC cases).

Diagnostic property	Control and cirrhotic cases	Cirrhotic and HCC cases
AUC (95% CI)	0.855 (0.715 - 0.995)	0.974 (0.936 - 1)
P value	0.002*	<0.001*
Cutoff point	>3980 pg/mL (cirrhotic)	9020
Sensitivity	84%	96%
Specificity	81%	92%
Accuracy	86%	97%

AUC: area under the curve.

5. Discussion

Hepatocellular carcinoma is one of the leading causes of death and usually has an aggressive course. Early detection and reliable, economical screening are needed. An optimal screening test is not available and many cases are missed to advanced stages with the current screening tools, to advanced stages.

This study was conducted to investigate the serum level of MidKine as a marker of HCC in HCV-related liver cirrhosis. The study included a total of 80 subjects divided into 3 groups; the healthy control group (10 patients), the cirrhotic group (20 patients), and the HCC group (50 patients).

In our study, the median level of AFP in the HCC group was 110 IU/mL which was higher than the median value in the control and cirrhotic groups with a high level of significance between the study groups ($P < 0.001$).

This is consistent with previously reported data on AFP in HCC patients. For example, Yang *et al.* (2016) showed that serum AFP levels were significantly higher in patients with HCC than in patients with cirrhosis and chronic hepatitis and in healthy controls [9]. This is also consistent with the findings of El-Edel and his colleagues, who showed that serum AFP levels were significantly elevated in chronic liver disease and were even more elevated in HCC cases, with a statistically significant difference ($P < 0.001$) [6]. Many other studies reached similar conclusions [3] [10].

The median level of MK in the control group of our study was 3018 pg/mL, the median level in the cirrhosis group was 5165 pg/mL, and the median level in the HCC group was 18,298 pg/mL with a high level of significance between the different study groups ($P < 0.0001$).

This was consistent with the study of Shaheen *et al.* who reported that the serum level MK was significantly increased in the HCC group compared to the cirrhotic and healthy control groups [11]. The study by El-Edel and his colleagues agreed with this and showed that the levels of MK were increased in the HCC groups compared with the cirrhotic groups and in the HCC groups compared with the control groups with a highly significant difference. However, there was no significant difference between the liver cirrhosis and control groups

in terms of MK levels [6].

Zhu *et al.* came to similar conclusions. This study included three independent cohorts with a total of 933 participants, including 388 HCC cases and 545 different control groups enrolled from different medical centers. The results showed that MK was significantly increased in both HCC tissues and serum samples [12].

In another study, a highly significant statistical difference was found between the mean serum level MK in patients with HCC compared to patients with liver cirrhosis and healthy controls ($P < 0.001$) [13].

In this study, the best cutoff point of MidKine to discriminate controls from cirrhotics was 3980 pg/mL with 84% sensitivity, 81% specificity and overall accuracy of 86%. The best cutoff point of MidKine for distinguishing cirrhotic from HCC cases was 9020 pg/mL with 96% sensitivity, 92% specificity, and an overall accuracy of 97%, with higher sensitivity and specificity, compared with AFP.

El-Edel and colleagues showed that the sensitivity of MK at a cutoff value of 6200 pg/mL was significantly higher than that of AFP at a cutoff value of 2002 pg/mL, and that the specificity of MK at the same cutoff value was significantly higher than that of AFP at a cutoff value of 2002 pg/mL [6].

This is consistent with Zhu *et al.* who found that the optimal cutoff value of MK was 6540 pg/mL. At a cutoff value of 6540 pg/mL, the sensitivity of MK for HCC diagnosis was 86.9%, which was much higher than that of AFP (51.9%) [12].

In another study, the best cutoff values for MK and AFP for distinguishing HCC cases from those with cirrhosis were 3870 and 88,500 pg/mL, respectively, with sensitivity (92.5% versus 40%), specificity (83.3% versus 96.7%), and accuracy (88.5% versus 68.2%). The sensitivities of MK were significantly higher than AFP at all levels (92.5% versus 62.5%, 40% and 25%) [11]. The study by Mashaly *et al.* found that MidKine had higher sensitivity at a cut-off value of 1680 pg/mL than AFP at a cut-off value of 200 pg/mL (81.82% versus 52.27%) [14].

El-Akel and colleagues showed that MK has a sensitivity of 91% and a specificity of 90% for the detection of HCC at an optimal cut-off value of 3400 pg/dL with an AUC of 0.94 compared to cirrhosis, while AFP has a sensitivity of 56% and a specificity of 90% at an optimal cut-off value of 2150 pg/mL with an AUC of 0.63 compared to cirrhosis [13]. On the other hand, Hung *et al.* (2011) found that MK had a sensitivity of 51% and a specificity of 60% at a cut-off value of 5000 pg/mL [15].

This difference in results may be attributed to differences in the population studied, HCC stages, tumor size, tumor pathology, and number of patients included in both studies. These differences justify the need for a large multicenter study to standardize the optimal cutoff points.

In this study, there was a statistically significant correlation between MK levels and serum AFP levels, tumor size, and tumor stage ($P < 0.001$).

This was in contrast to Shaheen *et al.*, who found no significant correlation between serum levels of MK and tumor size, number, or serum levels of AFP, and no significant correlation was found between serum levels of MK and BCLC stages [11]. This is an area that should be investigated more intensively in the future.

In our study, among HCC cases, AFP was positive in 35 cases and negative in 15 cases out of 50 patients (30%), MK was 100% positive in all HCC cases. In our study, the percentage of HCC cases with negative AFP was 30% (15 patients), while the percentage of cases with negative MidKine was 0%.

Mashaly *et al.* (2018) showed that among HCC cases, 21 of 44 patients (47.7%) were AFP negative. MidKine was positive in (80.9%) (17/21) of AFP-negative HCC patients [14].

Vongsuvanh *et al.* (2016) showed that in patients with HCC, 56.98% (n = 49/86) had normal AFP. Of these 49 patients with AFP-negative HCC, 59.18% (n = 29/49) had MK using the optimal diagnostic cut-off of 0.44 ng/mL [16].

These results indicate that a major limitation of using AFP for HCC surveillance is the rate of AFP-negative HCC. MK has better diagnostic performance than AFP and increases the diagnostic yield in AFP-negative HCC cases [17]. These results indicate the importance of MK in the diagnosis of HCC, especially in AFP-negative cases.

Vongsuvanh *et al.* (2016) investigated the diagnostic efficacy of MK in different HCC subgroups using AUROC. In the detection of early-stage HCC (BCLC 0-A), MK showed an AUC of (0.63; 95% CI 0.52-0.73) with a statistically significant predictive value ($P < 0.05$) [16].

Zhu *et al.* (2013) reported by ROC curve analysis that serum MK had a better performance compared with AFP in distinguishing early-stage hepatocellular carcinomas as well as small hepatocellular carcinomas. Even in very early-stage hepatocellular carcinomas, MK showed a higher sensitivity compared with AFP (80% vs. 40%) [12].

In our study, in the group with HCC, there were 30 cases with early HCC. Among the cases with early HCC, there were 4 cases negative for AFP (13.3%) while the percentage of cases with negative MidKine was 0%, indicating a better diagnostic value of MK in early HCC stages.

6. Conclusion

Hepatocellular carcinoma is a common complication of HCV-related cirrhosis. MidKine is a novel sensitive marker that could be utilized for the early detection of HCC. In addition to its diagnostic ability, MidKine is correlated with tumor stage and size and it has an important diagnostic utility in AFP-negative cases.

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Conflicts of Interest

Authors declare no conflicts of interest.

Ethics Approval and Consent to Participate

The study has been approved by the ethics committee of our university (Mansoura Faculty of Medicine, Mansoura University, Institutional Review Board. Informed written consent was obtained from all individual participants included in the study.

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