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Evaluation of Serum Midkine as a Marker of Hepatocellular Carcinoma in Cirrhotic Patients

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ABSTRACT

Background: Hepatocellular carcinoma (HCC) is the fifth most common malignancy. Midkine (MK) is a cytokine or a growth factor belongs to the carbohydrate-binding proteins. MK is over expressed in hepatocellular carcinoma. Furthermore, patients with high MK expression in the tumor frequently have a worse prognosis than those with low MK expression.

Objectives: The aim of the work was to evaluate serum midkine as a marker for Hepatocellular carcinoma in cirrhotic patients.

Patients and Methods: This study was conducted on 90 subjects who were divided into three groups: **group I** included 40 patients with liver cirrhosis and hepatocellular carcinoma, **group II** included 40 patients with HCV related liver cirrhosis without HCC and **group III** with 10 healthy subjects as controls. Plasma level of midkine was measured for all subjects.

Results: Serum levels of midkine were highest in patients of group I with HCC compared to those with liver cirrhosis and the control groups (p value< 0.001). Also midkine values increased with tumor number and overall size. According to the ROC curve, the best cutoff value for midkine differentiating HCC from liver cirrhosis cases was 8500pg/mL, above which the sensitivity to discriminate HCC = 100% and below which the specificity to discriminate liver cirrhosis = 87.5% with 94.5% accuracy.

Conclusion: Serum midkine level was significantly elevated in HCC patients, so it can be used as a diagnostic marker for HCC. Also, it was directly correlated to the tumor number and overall size so it has a good prognostic value.

Keywords: Hepatocellular carcinoma, Serum Midkine, Cirrhotic liver.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most frequent liver cancer [(1)]. HCC is the fifth most common cancer and the second leading cause of cancer-related deaths. HCC most often develops in patients with a history of cirrhosis due to chronic alcohol abuse, non-alcoholic fatty liver disease, or hepatitis C virus (HCV) infection [(2)].

Biomarkers that distinguish HCC from inflammation and cirrhosis are desperately needed in order to enhance prognosis of these patients. Contributing to the poor prognosis of HCC is the lack of specific symptoms in the early stages of the disease. More than 60% of patients are diagnosed with late-stage disease after metastasis has occurred [(3)], resulting in an overall 5-year survival rate of < 16% [(4)]. In contrast, patients diagnosed with early stage disease have a relatively good prognosis, with a 5-year survival rate of > 70% [(5)].

The diagnosis of HCC without a pathological diagnosis can be achieved by assessing serum α-fetoprotein (AFP) levels and diagnostic imaging, such as computed tomography (CT) and magnetic resonance imaging (MRI)[(6)].

The ideal HCC biomarker is one that enables clinicians to diagnose asymptomatic patients and can be widely used in a screening process. In general, a biomarker valuable for clinical use achieves a level of sensitivity and specificity of ≥ 90%, and is non-invasive and cost-effective to allow widespread use. The most desirable biomarker is therefore tumor-specific and easily detectable in bodily fluids, such as serum, plasma, and bile [(7)].

Midkine (MDK) is a heparin-binding growth factor that has been associated with tumor migration and proliferation [(8)]. Not surprisingly, MDK is often over expressed in various human tumors, making it an attractive target in tumor detection and treatment [(8)]. A clinical study on a cohort of 388 HCC patients and 545 hospital enrollees diagnosed with other diseases identified MDK as a discriminating tissue and serum biomarker with better sensitivity (86.9%, serum MDK) than AFP (51.9%)[(9)].

The aim of the current work was to evaluate serum midkine as a marker for Hepatocellular carcinoma in cirrhotic Patients.

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PATIENTS AND METHODS

This case control cohort study included a total of 90 ninety age and sex matched subjects, recruited from Internal Medicine Department and clinics at Ain Shams University Hospitals.

Ethical approval:

The steps of the study were explained, and written consent was taken from all patients.

Approval of the ethical committee was obtained.

The included subjects were divided into three groups; **Group 1** consisted of forty HCC patients diagnosed by abdominal CT with contrast, **Group 2** consisted of forty HCV Cirrhotic patients without HCC and **Group 3 (control)** consisted of ten apparently healthy subjects with no past medical history as a control group.

Exclusion Criteria:

1. Patient with other liver disease except HCV Ab +ve patients.
2. Patients with previous treatment for HCC (Either chemoembolization or radiofrequency).
3. Patients with expected elevated serum midkine level for non-hepatic cause including:
   - Dilated cardiomyopathy.
   - Uncontrolled hypertension.
   - Connective tissue disease as rheumatoid arthritis, ankylosing spondylitis, multiple sclerosis or systemic lupus erythematosis.
   - Psoriasis.
   - Chronic obstructive pulmonary disease.
   - Chronic pancreatitis.
   - Bipolar disorder.
   - Any other malignancy.
   - Thyroid nodules.

All subjects were subjected to:

- **Full history and clinical examination.**
- **Laboratory investigations including:** Complete blood count (CBC), kidney function tests and electrolytes (urea, creatinine, sodium (Na^+), potassium (K^+), liver function tests (Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Gamma glutamyltranspeptidase (GGT), total protein, albumin (Alb), total bilirubin (T. Bil.), Direct bilirubin (D. Bil.), Coagulation profile prothrombin time (PT), international normalization Ratio (INR), Partial thromboplastin time (PTT), Viral markers, Hepatitis C serum antibody using ELISA (HCV Ab) and Hepatitis B surface antigen (HBsAg), Urine analysis, prostatic specific antigen (PSA), serum midkine was measured by Enzyme Linked Immunosorbent assay (ELISA) technique and Serum Alpha Fetoprotein.
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- **Radiological examination:**
  - Plain X-ray chest, Pelvi-abdominal ultrasound, Triphasic CT or MRI abdomen.
  - ❖ Clinical staging of HCC was according to the Barcelona Clinic Liver Cancer (BCLC) system.
  - ❖ Cirrhosis was diagnosed by on the basis of clinical, laboratory and/or imaging evidence.

Sample preparation:

Blood samples were collected and divided between two tubes; ethylene diamine tetra acetic acid (EDTA) tube for CBC and plain tube for separated sera which stored at -80°C until testing, for measuring other biochemical parameter assays.

Statistical Methods

The SPSS 10.0 for windows was used for data management and analysis and the Microsoft power point for charts. Quantitative data were presented as mean +SD. For comparison of the two groups means, the Student's t-test was used, while for the comparison of the three groups' means, one way analysis of variance (ANOVA) was used followed by Post Hoc test. Non parametric quantitative data were expressed as median (range), Tukey's tests were used for comparison of means. Qualitative data was expressed as frequency and percentage. Association between qualitative data was done using Chi- square test. P value was considered significant at 0.05 while highly significant at <0.01 while non-significant at >0.05. The ROC was constructed to obtain the most sensitive and specific cutoff value for serum MDK in diagnosing HCC.

RESULTS

This case control study was conducted on ninety age and sex matched subjects with age range 25-73 year (51.8±6.74). They were divided into three groups, **Group I:** 40 HCC patients, **Group II:** 40 HCV patients without HCC, **Group III:** 10 healthy subjects as controls. There was no statistically significant difference between the three groups as regard age, gender, body mass index or smoking.

Table (1): Comparing the three groups regarding the laboratory data.

		Groups									ANOVA	
		Group I			Group II			Group III			F	P-value
Regarding the liver functions												
AST(U/L)	**Mean ±SD**	94.286	±	6.774	66.714	±	3.806	36.850	±	7.286	10.887	<0.001
ALT(U/L)	**Mean ±SD**	63.400	±	2.685	42.743	±	9.901	23.950	±	5.898	19.102	<0.001
T.bilirubin (mg/dl)	**Mean ±SD**	6.480	±	1.668	4.211	±	1.484	0.855	±	0.209	15.379	<0.001
D.bilirubin (mg/dl)	**Mean ±SD**	3.769	±	0.560	2.200	±	0.561	0.170	±	0.03	16.213	<0.001
T. proteins (g/dl)	**Mean ±SD**	6.794	±	0.760	6.380	±	0.446	7.185	±	0.436	12.401	<0.001
S. Alb (g/dl)	**Mean ±SD**	2.186	±	0.792	2.626	±	0.571	4.290	±	0.415	71.531	<0.001
INR	**Mean ±SD**	2.083	±	0.681	1.545	±	0.27	0.906	±	0.137	39.334	<0.001
Regarding the kidney functions												
Urea (mg/dl)	**Mean ±SD**	33.486	±	3.292	31.457	±	3.223	35.800	±	2.911	0.703	0.498
Creatinine (mg/dl)	**Mean ±SD**	1.443	±	0.037	1.337	±	0.08	0.832	±	0.207	4.029	0.02
Na (mEq/L)	**Mean ±SD**	130.971	±	4.091	133.286	±	5.062	139.350	±	2.346	25.484	<0.001
K (mEq/L)	**Mean ±SD**	3.817	±	0.684	3.609	±	0.497	4.175	±	0.665	5.430	<0.006
Regarding the CBC												
HB(g/dl)	**Mean ±SD**	9.949	±	1.561	9.914	±	1.288	13.430	±	0.900	53.561	<0.001
TLC (cell/mm^3)	**Mean ±SD**	5.791	±	1.366	5.700	±	1.619	6.895	±	1.354	4.803	<0.011
PLT/mcl	**Mean ±SD**	69.457	±	5.469	100.943	±	4.978	296.050	±	7.484	132.883	<0.001

This table revealed a high statistically significant difference between the three groups as regards AST, ALT, total bilirubin, direct bilirubin, total proteins, albumin, Na, K, INR, Hb, TLC and PLT (p value <0.001), also there was statistically significant difference as regard serum creatinine level (p value =0.02) . While there was no significant difference regarding BUN (p= 0.498).

Table (2): Comparison between the three groups as regards serum Midkine level

Groups	Serum Midkine			ANOVA	
	Mean	±	SD	F	P-value
Group I	11968.750	±	430.760	149.074	<0.001*
Group II	6309.375	±	66.865		
Group III	1015.000	±	16.272		
TUKEY'S Test					
I &II	**I & III**		**II & III**		
<0.001*	<0.001*		<0.001*		

As regard Midkine there was a highly statistical significant difference between the three groups. There is a high statistical difference of (p value<0.001) when comparing serum Midkine in groups I and II and I and III also when comparing groups II and III.

Table (31): Comparison between the three groups as regards alpha-fetoprotein.

Groups	Alpha fetoprotein (ng/ml)			ANOVA	
	Mean	±	SD	F	P-value
Group I	210.93	±	9.23	13.013	<0.001
Group II	8.48	±	1.95		
Group III	3.34	±	0.85		
TUKEY'S Test					
I&II	**I&III**		**II&III**		
<0.001	<0.001		>0.05		

Comparing the three groups as regards alpha-fetoprotein there was a highly statistical significant difference between them where the highest values were in group **I**. There was a highly statistical difference when comparing alpha fetoprotein in groups I and II and I and III while not when comparing groups II and III.

Table (4): Correlation between Midkine and all other parameters in group I.

Correlations

	Midkine	
	R	P-value
Age (Year)	-0.064	0.693
BMI (kg/m^2)	-0.102	0.533
Hb (g/dl)	0.155	0.341
Wbc (mcL)	-0.188	0.245
PLT (mcL)	0.151	0.352
ALT (U/L)	-0.110	0.498
AST (U/L)	-0.189	0.243
T BIL (mg/dl)	-0.247	0.124
D BIL (mg/dl)	-0.244	0.129
T.proteins (g/dl)	0.044	0.787
Albumin (g/dl)	0.209	0.195
INR	-0.112	0.491
BUN (mg/dL)	0.042	0.799
Cr (mg/dL)	0.076	0.642
Na(mEq/L)	0.049	0.764
K(mEq/L)	0.160	0.323
AFP (ng/mL)	0.719	<0.001*
Size of F Lesions (cm)	0.457	0.003*

Correlating Midkine level with all other parameters in the HCC group showed that its value had a highly positive significant correlation with AFP level (p< 0.001) and also we found significant positive correlation with overall size of hepatic focal lesions (p< 0003).

Table (5): Correlation between Midkine and number of HCC lesions.

N. of focal Lesions	Serum Midkine			ANOVA	
	Mean	±	SD	F	P-value
One	11358.333	±	479.674	4.000	0.009*
Two	11545.455	±	254.084		
Three	12156.250	±	552.468		
Four	12937.500	±	95.495		
Multiple	13359.375	±	79.009		

TUKEY'S Test				
	One	Two	Three	Four
Two	0.996			
Three	0.788	0.917		
Four	0.461	0.602	0.950	
Multiple	0.007*	0.028*	0.526	0.993

Correlating midkine level with number of focal lesions shows a significant positive correlation (p= 0.009) where midkine level is higher as number of HCC lesions increases.

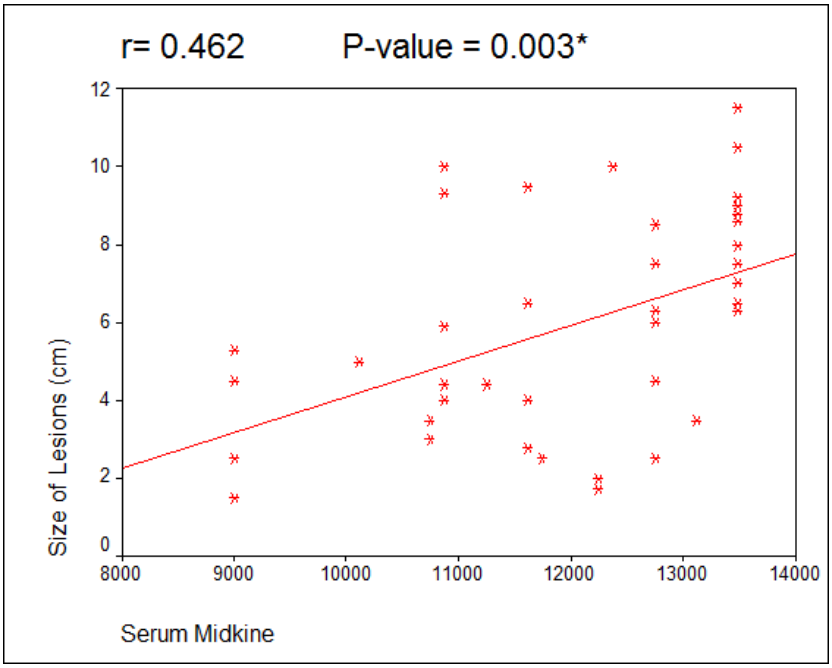

Figure (1): Correlation between Midkine and tumor size.

As regards the tumor size positive correlation was also seen between midkine and size of focal lesion, where midkine level increase as the tumor size increases with p value less than 0.003*

Table (6): Correlation between Midkine and different BCLC stages.

BCLC	Serum Midkine			ANOVA	
	Mean	±	SD		-value
BCLC A	12475.000	±	83.511	0.641	0.594
BCLC B	11875.000	±	555.233		
BCLC C	11953.125	±	646.069		
BCLC D	11613.636	±	646.449		

There was no significant correlation between serum midkine levels and BCLC stages.

Table (7): Correlation between Midkine and portal vein invasion.

PV	Serum Midkine			T-Test	
	Mean	±	SD	T	P-value
Patent	12043.269	±	228.282	0.444	0.659
Thrombosed	11830.357	±	791.142		

There was no significant correlation between serum midkine and portal vein invasion.

Table (8): Correlation between Midkine and Child Pugh score classification.

Child Pugh	Serum Midkine			ANOVA	
	Mean	±	SD	F	P-value
Child A	11861.111	±	527.667	0.643	0.532
Child B	12212.500	±	285.099		
Child C	11613.636	±	646.449		

There was no significant correlation between serum midkine and Child Pugh classification.

Table (9): ROC curve between group A and group B

ROC curve between group A and group B					
Cutoff	Sens.	Spec.	PPV	NPV	Accuracy
>8500	100.0	87.50	88.9	100.0	94.5%

ROC curve was performed for the best cutoff point to differentiate between HCC group and cirrhotic group. According to the curve, the best cutoff value for midkine differentiating HCC from liver cirrhosis cases was 8500pg/mL, above which the sensitivity to discriminate HCC = 100% and below which the specificity to discriminate liver cirrhosis = 87.5% with 94.5% accuracy.

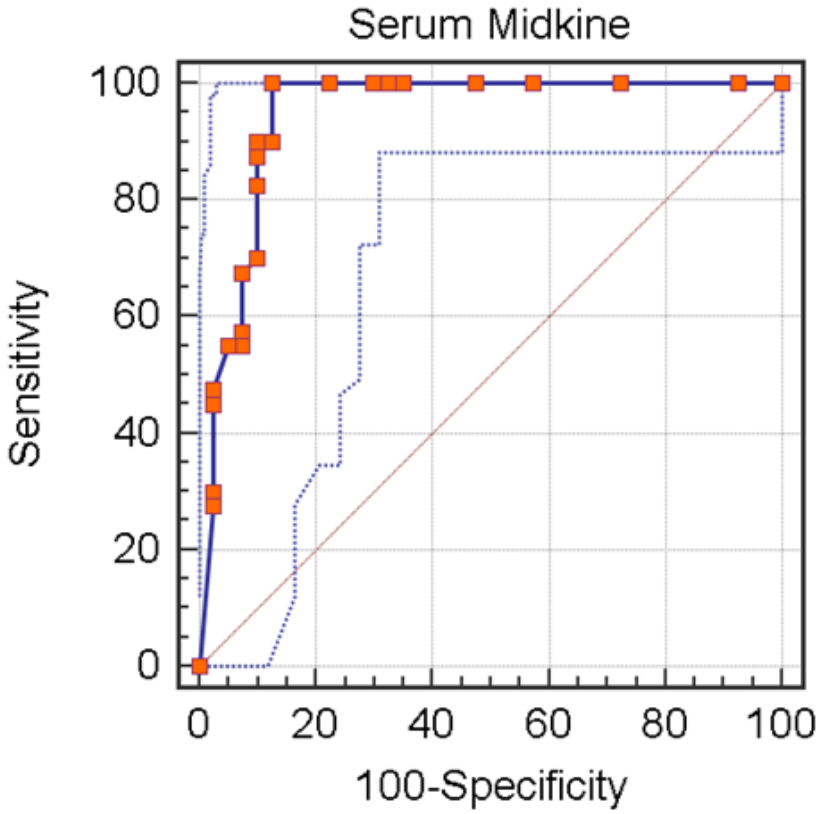

Figure (2): ROC curve of serum midkine between group A and group B.

DISCUSSION

In this study was aimed to evaluate the serum midkine level in patient with hepatocellular carcinoma and liver cirrhosis secondary to HCV infection and compared with healthy control.

In the present study the age of the patient with HCC ranged between 46 - 73 years with a mean 55.8 ±5.77 years which was consistent with **Johnson** [10] who found that the average age of patients ranged from fifth to sixth decades of life.

As regard laboratory parameters between the three groups, it showed a highly significant difference in serum levels of AST, ALT, albumin, bilirubin, PT, platelet count between the 3 groups with increased severity of liver functions tests in HCC group more than the other groups and this was in agreement with **Dooley *et al.*** [11]**.**

Regarding the serum levels of AFP in the current study, there was a highly significant difference between patients with HCC and those with liver cirrhosis where the mean was 210.93 ng/ml in patients with HCC and 8.48 ng/ml ml in patients with liver cirrhosis with a p value <0.001, this was in agreement with **Liu *et al.*** [12] who stated that AFP levels significantly differed in patients with HCC having a mean 250.65 nm/ml and patients with liver cirrhosis with a median 2.32ng/ml and p value <0.001.

Concerning the value of Midkine in diagnosing HCC, there was a highly significant difference in its values in patients with HCC over liver cirrhosis where in HCC the values ranged between 9000-13500 pg/ml and mean 11968.750 ±1430.760 pg/ml compared to 3000 -13500 pg/mL, a mean of 6309.375 ± 2666.865 pg/ml in cirrhotic over 750 - 1600 pg/mL and a mean 1015.000 ± 316.272 pg/ml in healthy controls with a P value < 0.001 indicating the highest values in HCC patients.

Our results are close to those reported by **Zhu *et al.*** [9] that study involved three independent cohorts with a total of 933 participants including 388 HCC cases and 545 different controls enrolled from different medical centers. Results showed that MDK levels were significantly elevated in HCC tissues as well as serum samples; serum MDK at the cutoff value of 0.654 ng/mL for HCC diagnosis showed high sensitivity 86.9% with specificity 83.9%.

But in our study ROC curve was performed for the best cutoff point to differentiate between HCC group and cirrhotic group using MDK. According to the curve, the best cutoff value for MDK differentiating HCC from cirrhotic cases was 8500 pg/mL (8.5 ng/ml), above which the sensitivity to discriminate HCC = 100% and below which the specificity to discriminate liver cirrhosis is 87.5% with 94.5% accuracy.

Another study by **Zhu *et al.* [9]** the median serum MDK level in hepatocellular carcinomas (1.204 ng/mL) was significantly elevated compared with that in healthy individuals (0.195 ng/mL) and patients with different types of liver diseases (0.739 ng/ml, $P < 0.05$) in patients with benign liver tumors; 0.265 ng/mL, ($P < 0.0001$) in patients with liver cirrhosis.

Concerning correlation between levels of midkine with different tumor characteristics, in our study, there was a significant positive correlation between midkine values and tumor number with (P value 0.009). Correlation between midkine values and tumor size, there was positive significant correlation with (P value < 0.003). There was no significant correlation between Midkine level and macro vascular invasion with (P value 0.659). As regards child score classification and BCLC classification, there was no significant correlation between Midkine and child score or with BCLC score where p value was 0.532 and 0.594 respectively.

CONCLUSION

Serum midkine could be used as a marker for diagnosis of HCC with cut off value 8500 pg/mL, above which the sensitivity to discriminate HCC = 100% and below which the specificity to discriminate liver cirrhosis = 87.5% with 94.5% accuracy. Also, it has good prognostic value as it is significantly directly correlated with tumor number and overall size.

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