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Value of Midkine as a Diagnostic Serum Marker in Hepatocellular Carcinoma

Desouky E. Abou Ammo [1] , Ashraf F. Barakat [2], Amira Y. Ahmad [1] , Rehab R. Farag [1]
1- Department of Clinical Pathology, 2- Department of oncology, Faculty of Medicine, Tanta University
Corresponding author: Rehab R. Farag, email: Rehab.ramadan1988@gmail.com

ABSTRACT

Background: hepatocellular carcinoma (HCC) represents a global health problem. It is the fifth most common cancer but the second leading cause of cancer-related death in men worldwide. In Middle Eastern countries, liver cancer is a major concern among men, especially in Egypt and Saudi Arabia. The incidence of HCC has increased sharply in the last 5–10 years, with an especially high incidence in Egypt. So, early detection and diagnosis of these cases are required for successful treatments and improved outcomes. **Aim of the Work:** this study aimed to detect the efficacy of serum level of Midkine as an early marker for HCC diagnosis compared to AFP serum level. **Patients and Methods:** this prospective study was conducted on a total of 50 subjects, 30 of them with HCC and 20 apparently healthy subjects matched for age and sex with patients. It was carried out at the Clinical Pathology Department, Tanta University Hospital. **Results:** a significant increase ($p < 0.001$) in serum midkine level was detected in HCC group compared with control group from a mean of 391.45 ± 96.09, in control group to a mean of 1074.53 ± 106.27, in HCC. **Conclusion:** serum midkine may serve as a novel diagnostic tumor marker for the detection of hepatocellular carcinomas. **Recommendations:** it was recommended to do further studies on early cases with larger population including AFP-negative patients, to justify its implementation in clinical practice.

Keywords: hepatocellular carcinoma, midkine, alpha-fetoprotein.

INTRODUCTION

Hepatocellular carcinoma (HCC) represents a global health problem. It is the fifth most common form of cancer worldwide and the third most common cause of cancer-related deaths [(1)]. In Egypt, HCC represents 75% of malignant liver tumors. Liver cancer is the 5th most common cancer in both genders, the 6th in female representing 3.4% of cancers and 2nd in order in males after cancer urinary bladder representing 11.5% of all cancers. In 2010, liver cancer came in the 3rd order in both sexes (8.1%), 1st in males (12.1%) and 5th in females (4%) [(2)]. Owing to the diagnostic and therapeutic progress during the past decades, the hepatocellular carcinoma outcome has been improved in a proportion of patients who were diagnosed at an early stage and received curative treatments [(3)]. However, only about 10% to 20% of patients are currently eligible for potentially curative therapies at the time of diagnosis [(4)]. Most of the patients with hepatocellular carcinomas are diagnosed at an advanced stage and their prognosis remain very poor [(5)]. Thus, early detection and diagnosis of hepatocellular carcinomas still present the best chance for successful treatments and improved outcomes [(6)].Alpha-fetoprotein (AFP) has been widely used as a serologic diagnostic tumor marker for hepatocellular carcinomas. However, serum AFP is elevated in only about 33% to 65% of small hepatocellular carcinomas and nonspecific elevation of serum AFP has been found in 15% to 58% of patients with chronic hepatitis and 11% to 47% of liver cirrhosis [(7)]. Midkine, a small heparin-binding growth factor, was originally discovered in embryonal carcinoma cells and involved in the early stage of retinoic acid–induced differentiation [(8)]. Midkine was identified as 1 of the 5 important potential novel biomarkers for early detection of hepatocellular carcinomas [(9)]. In addition, mounting evidence has indicated that midkine plays a significant role in carcinogenesis- related activities, such as proliferation, migration, anti-apoptosis, mitogenesis, transformation, and angiogenesis, in many types of solid tumors, including hepatocellular carcinomas [(10)].

AIM OF THE WORK

This study aimed to detect the efficacy of serum level of Midkine as an early marker for HCC diagnosis compared to AFP serum level.

SUBJECTS AND METHODS

This prospective study was carried out at Clinical Pathology Department, Tanta University Hospital and included 50 subjects categorized into two groups:

- **Group I**: 30 HCC patients, selected from Clinical Oncology Department Tanta University Hospital.
- **Group II:** 20 apparently healthy subjects matched for age and sex with patients and had included as a control group.

Studied groups were subjected to the following:

1. Full history taking and thorough clinical examination.

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2. Abdominal ultrasonography and ultrasound guided liver biopsy for the cirrhotic patients when possible.
3. Triphasic C.T for patients with focal lesions.
4. Laboratory investigations including:

a. Liver function tests included:
- Alanine aminotransferase (ALT),
- Aspartate aminotransferase (AST),
- Albumin,
- Prothrombin time (PT).

b. Complete blood count.
c. Viral hepatitis markers.
d. Serum AFP level.
e. Serum Midkine level using the enzyme-linked immunosorbent assay (ELISA) kit supplied by Sunred Biological Technology Combany; China

All data were recorded in Excel XP for windows. Statistics were performed using SPSS 23.0 for Windows. For comparison of the means of two groups, Student's t-test was used and a $P < 0.05$ was considered as significant.

The study was approved by the Ethics Board of Tanta University.

RESULTS

In this study, there were 50 subjects (30 HCC patients, and 20 apparently healthy subjects). Male/female ratios were 21/9, and 14/6, respectively. The mean age was 56.8±8.56 in HCC and 55.05±6.46 in the control group. There was statistically significant increase in the serum levels of midkine, AFP, AST, ALT, and PT in HCC patients, compared to control group ($p< 0.001$). Whereas there was statistically significant decrease in the serum level of albumin, hemoglobin and platelet count in HCC patients compared to control group ($p< 0.001$).The demographic features and laboratory values and their statistical significance are given in **Table 1.**

Table 1: the demographic features and laboratory levels of all patients

	HCC	*Control*	*P-value*
Male/female ratio	21/9	14/6	
Age (years)	56.8±8.56	55.05±6.46	
Hemoglobin (g/dL)	10.63±1.54	13.29±0.99	<0.001*
White blood cell ($\times10^3$/mm^3)	4.73±1.44	5.43±0.96	0.0635
Platelet($\times10^3$/mm^3)	128.57±34.12	176.85±15.90	<0.001*
Prothrombin time (sec)	14.16±2.20	10.61±1.15	<0.001*
AST (U/L)	41.50±9.62	16.90±3.51	<0.001*
ALT (U/L)	44.07±10.92	17.45±3.95	<0.001*
Albumin (g/dL)	3.41±0.45	4.66±0.56	<0.001*
AFP (ng/mL)	859.60±210.16	4.45±1.10	<0.001*
Midkine (ng/L)	1074.53±106.27	391.45±96.09	<0.001*

Diagnostic performance of AFP and midkine

The diagnostic performance of AFP and midkine were done using ROC curve as shown in **table 2**, The cutoff value of AFP was 208.5 (ng/ml), showed a diagnostic sensitivity of 100%, specificity 100%, positive predictive value 100% and negative predictive value 100%.The area under the curve (AUC) was 1.000 as shown in **figure 1.**

The cutoff value of midkine was 712.5 (ng/l), showed a diagnostic sensitivity of 100%, specificity 100%, positive predictive value 100% and negative predictive value 100%.The area under the curve (AUC) was 1.000 as shown in **figure 2.**

Table 2: diagnostic performance of serum AFP (ng/ml) and serum midkine (ng/l) discriminating group I from group II

ROC curve between group I and group II as regard serum AFP					
Cut off	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy**
208.5 (ng/ml)	**100 %**	**100 %**	**100 %**	**100 %**	**1.000**
ROC curve between group I and group II as regard serum midkine					
Cut off	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy**
712.5 (ng/l)	**100 %**	**100 %**	**100 %**	**100 %**	**1.000**

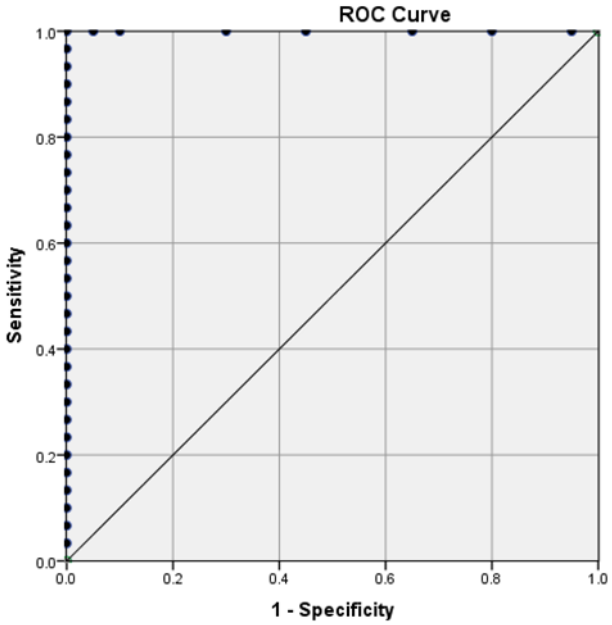

Figure 1*:* diagnostic performance of serum AFP (ng/ml) discriminating group I from group II

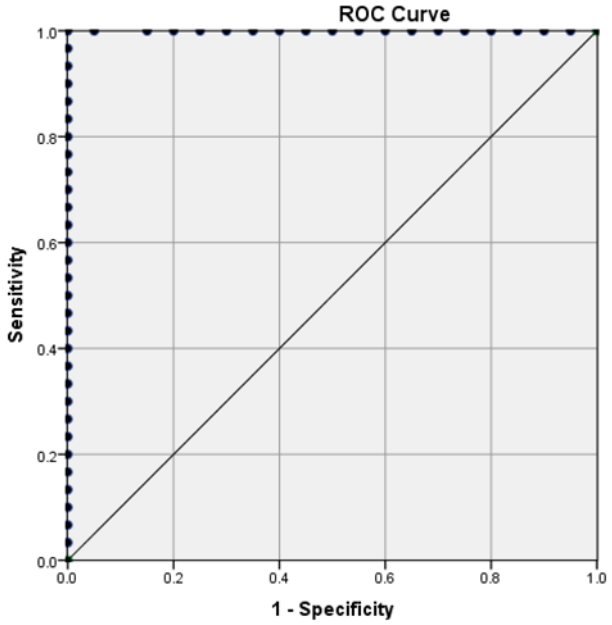

Figure 2: diagnostic performance of serum midkine (ng/l) discriminating group I from group II

DISCUSSION

The present study revealed a statistically significant increase in the serum levels of liver enzymes, AST and ALT, in HCC group compared to control group. This finding is in agreement with those of **Panteghini** who explained that result is due to the leakage of hepatic enzymes through its inflamed wall [(11)]. Serum ALT and AST are released from damaged hepatocytes into blood and their activities have been widely recognized as effective tools to detect liver diseases [(12)]. Actually, ALT is the most extensively investigated serum enzyme and elevated ALT has been associated with the mortality of various liver diseases [(13)].Previous observation had shown a correlation between AST/ALT ratio and presence of liver cirrhosis [(14)]. The present study revealed also a statistically significant increase in the PT and statistically significant decrease in the serum level of albumin, in HCC patients compared to control group. This could be attributed to the impaired synthetic ability of the liver for albumin and vitamin K, the co-factor of extrinsic coagulation pathway [(15)].

But, only 5 % of the daily amount of albumin needed is synthesized by the liver [(16)], indicating that serum albumin level is affected by multifactorial situations, other than the decrease in the liver's ability to synthesize it, like anorexia-related malnutrition and muscle loss due to liver

cirrhosis and cancer cachexia [17]. **Don and Kaysen** reported that hypoalbuminemia, which is one of the important laboratory findings of cancer cachexia, may be reduced with a multifactorial pathogenesis in hepatocellular carcinoma, and symptoms of inflammatory response and/or cancer cachexia cannot be excluded in advanced-stage hepatocellular carcinoma [18]. The present study revealed also a statistically significant decrease in the hemoglobin and RBCs count, in HCC patients compared to control group and indicating presence of anemia in HCC patients. Anemia in cancer patients is a multifactorial event and can be caused by chemotherapy, bone marrow replacement by metastasis, hemolysis or nutritional deficiencies [19]. Other suggested reasons are defects in iron reutilization from bone marrow cells; shortened survival time of red cells, decreased erythropoiesis caused by hypoproliferative bone marrow or decreased erythropoietin (EPO) production [20].

The present study revealed also a statistically significant decrease in the platelet count, in HCC patients compared to control group. The presence of thrombocytopenia in HCC patients probably reflects the severity of the portal hypertension which is secondary to the cirrhosis in which HCC usually develops [21]. Thrombocytopenia-associated HCC has been shown to be associated with smaller-size tumors [22]. In contrast, several reports have shown that large-size HCCs often have normal platelet counts, likely due to less portal hypertension [23]. **Carr and Guerra** found that thrombocytosis in association with HCC occurs in patients with larger tumor sizes and better liver function [23].The results of AFP in our study showed that serum AFP was significantly higher in HCC group when compared to control group. Also, midkine was significantly higher in HCC group when compared to the control group. This is in agreement with **Wei *et al***. who showed that serum AFP was significantly higher in HCC group when compared to liver cirrhosis group or control group and referred that to the increase in selective transcriptional activation in AFP gene in the malignant hepatocytes resulting in increased secretion of AFP during the development of HCC to inhibit immune response of liver cancer cells [24].

Shaheen *et al.* found that serum midkine was significantly elevated in patients with hepatocellular carcinomas compared with the healthy controls. In addition, serum midkine was not significantly higher in the liver cirrhosis group than that in healthy group, in contrast to serum AFP which was significantly elevated in the liver cirrhosis group when compared to healthy group. This means that the well-known nonspecific elevations of AFP in patients with liver cirrhosis were not significantly elicited with serum midkine increasing its specificity as a novel diagnostic marker for HCC [2].

Comparison of midkine results in HCC patients versus healthy subjects demonstrated 100% sensitivity as well as 100% specificity over a cutoff value of 712.5 ng/l. This is considered as an excellent discrimination power for diagnostic use of midkine. Similarly, AFP results in HCC patients versus healthy subjects showed also 100% sensitivity as well as 100% specificity at a cutoff value of 208.5 ng/ml. On comparing the sensitivities and specificities of midkine to those of AFP, **Vongsuvanh *et al.*** found that midkine and AFP were significantly associated with HCC diagnosis and from which the following equation was derived: 3*log AFP + log midkine. When this combined score was compared to AFP in HCC diagnosis, the AUC was only marginally improved compared to AFP alone (0.846 vs 0.831), suggesting that combining biomarkers did not significantly improve the diagnosis of HCC compared to either test alone [25]. While, **Shaheen *et al.*** found that the AUC of combined midkine and AFP for discrimination between HCC and liver cirrhosis patients was larger than that midkine alone (0.963 versus 0.941), suggesting that combination of midkine and APF may be a promising strategy for early diagnosis of hepatocellular carcinomas [2].

Shaheen *et al.* reported that at the cutoff 0.387 ng/mL, the sensitivities of midkine were significantly higher than those of AFP at cutoff 20, 88.5, and 200 ng/mL (92.5 versus 62.5, 40, and 25% respectively), with similar specificities to AFP at cutoffs 88.5 and 200 ng/mL, while midkine had significantly higher specificity than that of AFP only at the value of 20 ng/mL (83.3 versus 53.3). These indicate that midkine is a novel marker and superior to AFP with a lower false positive rate in diagnosing hepatocellular carcinomas [2].

The role of serum midkine in AFP-negative (<20 IU/ml) HCCs was investigated by **Vongsuvanh *et al.***, who found that, in patients with HCC, 56.98% (n = 49/86) had normal AFP. Of these AFP-negative HCC patients, 59.18% (n = 29/49) had elevated midkine. Using a criteria of AFP $\geq$ 20 IU/ml or midkine $\geq$ 0.44ng/ml, a significantly greater number (76.7%; n = 66/86) of HCC cases were detected, supporting a complementary role of midkine to AFP in HCC diagnosis [25].

CONCLUSION

The results of the current study indicated clearly that serum midkine is a promising sensitive and specific tumor marker that could be added to the current standard tests for diagnosis of HCC in order to detect the disease at an early stage and hence improving the prognosis and survival rate of the patient. From these results, we can conclude that serum midkine may serve as a novel diagnostic tumor marker for the detection of hepatocellular carcinomas.

RECOMMENDATIONS

Further studies on early cases with larger population including AFP-negative patients are needed to justify its implementation in clinical practice.

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