Egypt. J. Chem. **Vol. 65**, No. 3 pp. 637 - 645 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Evaluation of Serum Midkine as a Diagnostic Marker for Hepatocellular Carcinoma in Hepatitis C Virus Patients with Liver Cirrhosis

Mohammed Kamel Sheata[1], Ashraf Abdel-Megid ElFakhry [2], Abdel Moneim El Torgoman [3], Samah El-Ghlban [4]

[1] Biochemistry Division, Department of chemistry, Faculty of science, Mansoura University, Egypt
[2] Department of Internal Mediciene, Faculty of Medicine, Mansoura university, Egypt
[3] Department of Organic Chemistry , Faculty of science, Menofia university, Egypt
[4] Biochemistry Division, Chemistry Department, Faculty of science, Menofia university, Egypt

Abstract:

Background cancer in the world. Its incidence is increasing worldwide reaching half million cases each year. The primary marker for HCC is Alpha Feto Protein (AFP) which is not secreted in all cases of HCC and may be normal in as many as 40% of patients with early HCC. Midkine(MK) is a heparin-binding cytokine or growth factor, promoting survival, growth, migration, gene expression and other activities of target cells. MK is over expressed in hepatocellular carcinoma. The aim of the study is to evaluate the diagnostic value of MK as a tumor marker of HCC. This study was conducted on 85 patients who were classified into three groups: Group I (HCC) group included 45 patients with HCC. Group II (Liver cirrhosis) group included 30 patients without any evidence of hepatic focal lesions as excluded by ultrasonography and AFP estimation. Group III (control group) included 10 normal people. The level of AFP and MK were estimated for all cases together with full clinical assessment liver biochemical profile, viral markers, conventional US and triphasic abdominal CT for HCC cases. Serum AFP median level was significantly (P <0.05) elevated in the HCC group (116 ng/ml) when compared with both control (2.5 ng/ml) and liver cirrhosis (3.7 ng/ml) groups. Midkine median level was also significantly(P <0.05) elevated in the HCC group (538 ng/I) when compared with both the control (238 ng/I) and liver cirrhosis (292.5 ng/I) groups. There were no significant differences (P <0.05) were found in MK concentration between males and females in each studied group. Serum midkine level was significantly elevated (P <0.05) in HCC patients, so it can be used as a diagnostic marker for HCC.

Keywords: Alfpha feto protein, Inflammation, Hepatitis-B.

1. INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the ten most commonly occurring cancers worldwide and it is the second cause of death from malignancy [(1)]. Each year half million new cases are diagnosed world –wide with disease burden highest in developing countries (85%of all cases) [(2)]. Mostly HCC develops in patients with history of chronic hepatitis or cirrhosis. Coexis- tance of inflammation& cirrhosis makes the early diagnosis of HCC more difficult [(3)]. HCC mortality rate increased by 40% as a result of hepatitis C virus (HCV) events [(4)]. Most patients with HCC are diagnosed at late stage, making prognosis of HCC very poor with five-year survival rate of less than 5% [(5)].

Although Alpha feto protein (AFP) is a widely used biomarker for the diagnosis of HCC, it is positive only in 60%-80% of cases, also false-positive make it difficult to distinguish early-stage HCC from other disorders, such as acute hepatitis and cirrhosis, as well as embryonic tumors and certain gastrointestinal tumors. Thus, additional biomarkers are needed to improve the diagnostic accuracy for HCC [(6)].

MK promotes growth, survival, migration and gene expression of various target cells [(7)]. It seems to play an important role in tumorigenesis and tumor invasion by enhancing the growth, survival and angiogenic activity of tumor cells [(8)].

Levels of MK were reported to be elevated in peripheral blood samples from patient with HCC and its level was low in chronic hepatic disease [(9)].

2. SUBJECTS AND METHODS

Study Design:

This is a prospective study that was conducted to evaluate serum Midkine as a marker for hepatocellular carcinoma in HCV-related liver cirrhosis.

*Corresponding author e-mail: s_elghlban@yahoo.com; (Samah I. El-Ghlban)
EJCHEM use only: Receive Date: 08 July 2021, Revise Date: 08 August 2021, Accept Date: 09 August 2021
DOI: 10.21608/ejchem.2021.84948.4140

Setting:
Patients were recruited from the specialized medical hospital at Mansoura Faculty of medicine between September 2019 to December 2020.
Study Population
This study was conducted on 75 chronic liver disease patients. Their diagnosis was based on clinical, laboratory and radiological bases. All patients gave informed consent to their participation in this study.

The patients were classified into three groups:

- Hepatocellular carcinoma **(HCC)** group : 45 patients (35 males (74.6%) and 10 females (25.4)) with their ages ranged from 42 to73 years (mean 61.1 y ± 5.5).
- Liver cirrhosis group: this group included 30 patients (15 males (50%) and 15 females (50%)), their ages ranged from 43 to 71 years (mean 61.2 y± 5.5).
- Control group : this group included 10 patients (5 males 50% and 5 females 50%), their ages ranged from 35 to 57 years (mean 59.3 y± 5.4).

Ethical consideration:

- The whole study design was approved by the ethical committee at Mansoura Faculty of Mediciene.
- Confidentiality and personal privacy were respected in all levels of the study.
- Patients feel free to withdraw from the study at any time without any consequences.

Patients were subjected to the following:

- Detailed history: including personal data (name, age, sex) and history of medical disease associated.
- Complete Clinical examination.

Inclusion criteria of cases included:
1. Age above 18 years
2. Both genders
3. Positive HCV patients were confirmed by PCR tests
4. cirrhotic patients diagnosed by clinical, biochemical, and abdominal ultra-sonographic Findings.
5. Patients suffering from HCC diagnosed with ultrasound and CT.

Exclusion criteria of cases:
1. Age below 18 years.
2 .Co- infection with Hepatitis-B virus or Hepatitis-I virus.
3. Prior liver transplant.
4. Patients who suffer from another type of cancer rather than HCC.
5 .Patients with previous TACE or RFA.

Radiological investigation: using England Ultrasonography

A. Abdominal US: Liver, spleen, portal vein and/or ascites.
B. Triphasic CT abdomen (for diagnosis of HCC regarding size and number of lesions).

Laboratory investigation:

- Liver function tests (serum albumin, serum bilirubin, prothrombin time (INR), serum creatinine, Alanine aminotransferase& Aspartate aminotransferase).
- Anti HCV and HCV PCR.
- Serum level of Alpha feto protein.
- Serum level of Midkine by ELISA with NOVA company(No 18,Keeyuan Road, Daxing Industry Zone, Beijing, China.

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 0.05. The ROC was constructed to obtain the most sensitive and specific cutoff value for serum MK in diagnosing HCC.

3. RESULTS

Table (1): Age and sex in the study groups

Parameter	**Group**			**Test of significance**	
	Control	Cirrhosis	HCC	F / χ^2	P value
Age	59.3 ± 5.4	61.2 ± 5.1	63.1 ± 5.5	F = 0.540	0.585
Sex N (%)				χ^2 = 0.746	0.689
Male	5 (50%)	15 (50%)	35 (74.6%)		
Female	5 (50%)	15(50%)	10 (25.4%)		

P value: One-Way ANOVA for age, and Chi-Square test for sex. There was no statistically significant difference in age among the study groups. - There was male dominance in the Hepatocellular carcinoma group (HCC).
- There was no statistical significant difference in sex among the cirrhotic,control groups.

Table (2): Laboratory parameters in the three groups

Parameter	Control	Cirrhosis	HCC	P value
One-Way ANOVA test				
Hemoglobin (gm%)	14.3± 1.8b	12.1±1.3 a	10.4± 2.3a	<0.001
Albumin (g/dl)	4.3±.3.6a	3.8± .18b	2.8± .51c	<0.001
Creatinine (mg/dl)	0.73± .11a	0.78± .2a	0.95± .27b	0.004
Kruskal-Wallis H-test				
ALT (IU/L)	23.5 (19-28) a	37.5(29-85.3)b	43(28-72)b	0.002
AST (IU/L)	24.5(18.5-29.3)a	31(26-57.3)a	58.5(37.5-94.5)b	<0.001
WBCs count (10^3)	6.3(5.3-6.7)	6.5(4.9-8.3)	5.6(4.8-6.4)	0.296
Platelet count (10^3)	232.5(167.5-332.5)a	80(71-104.3)b	72(60-86)b	<0.001
Total bilirubin (mg/dl)	0.48(.30-.71)a	1.0(.79-1.1)b	1.2(.80-1.2)b	<0.001
Direct bilirubin (mg/dl)	0.28(.23-.35)	0.22(.20-.43)	0.30(.20-.50)	0.532
INR (IQR)	1.04(1.0-1.12)a	1.1(1.0-1.2)b	1.2(1.1-1.3)b	<0.001
AFP (ng/ml)	2.5(1.9-3.5)a	3.7(3.1-5.7)a	116(20.2-485)b	<0.001
Midkine (pg/ml)	238(174-375)a	292.5(234-488)a	538(269.6-919.5)b	0.002

Data are described as mean SD in case One-way ANOVA test and median in case Kruskal-Wallis H-test. Values within a row with different superscribts differ significantly at $p<0.05$.

This table showed a statistically significant difference between the study groups as regards hemoglobin, albumin, creatinine, ALT, AST, platelet count, total bilirubin, and INR but not for WBCs count and direct bilirubin.

Table (3): Correlation between AFP and lab parameters

Lab parameter	Correlation coefficient	P value
ALT(IU/L)	0.245	0.032
AST(IU/L)	0.424	<0.001
Hemoglobin(gm%)	-0.252	0.027
WBC(10^3)	-0.107	0.355
Platelet count(10^3)	-0.443	<0.001
Total bilirubin (mg/dl)	0.416	<0.001
Direct bilirubin (mg/dl)	0.178	0.133
Albumin(g/dl)	-.488	<0.001
INR(IQR)	0.471	<0.001
Creatinine(mg/dl)	0.252	**0.029**

P value: Spearman's correlation test

This table showed a statistically significantly positive correlation between AFP and ALT, AST, Total bilirubin, Direct bilirubin, INR and creatinine and a statistically significantly negative correlation between AFP and Hemoglobin, WBC, platelet count and albumin

Table (4): Correlation between Midkine and lab parameters

Lab parameter	Correlation coefficient	P value
ALT(IU/L)	0.126	0.267
AST(IU/L)	0.293	0.008
Hemoglobin(gm%)	-0.198	0.080
WBC($10^{3)}$	-0.042	0.710
Platelet count($10^{3)}$	-0.015	0.894
Total bilirubin(mg/dl)	-0.063	0.597
Direct bilirubin(mg/dl)	-0.040	0.740
Albumin(g/dl)	-0.241	0.034
INR	0.190	0.097
Creatinine(mg/dl)	0.067	0.569

P value: Spearman's correlation test.

This table showed a statistically significantly positive correlation between midkine and ALT,AST, INR and Creatinine and a statistically significantly negative correlation between midkine and albumin, Hemoglobin, WBC, platelet count, Total bilirubin and Direct bilirubin.

Table (5): Correlation between BCLC stage and Biomarkers (AFP / Midkine)

Biomarker	r_s value	P value
AFP(ng/ml)	0.075	0.631
Midkine(pg/ml)	0.024	0.874

r_s: Spearman's correlation coefficient. P value: Spearman's correlation test.

This table showed no statistically significant correlation between BCLC stages and studied biomarkers (AFP and Midkine).

Table (6): Biomarkers' levels in different BCLC stages

Biomarker	Stage 0	Stage A	Stage B	Stage C	P value
N	4	12	22	5	
AFP(ng/ml)	95 (390.4)	79.5 (532.3)	163.5 (754.2)	116 (128.6)	0.616
Midkine(pg/ml)	530.5 (904.4)	370 (596.3)	577 (960.6)	327 (235.5)	0.338

Data: Median (IQR). P value: Kruskal-Wallis H-test.

This table showed no statistically significant difference in AFP and Midkine levels between different BCLC stages .

Table (7): Correlation between Milan criteria (within / outside) and Biomarkers (AFP / Midkine)

Biomarker	r_{pb} value	P value
AFP(ng/ml)	-0.194	0.213
Midkine(pg/ml)	-0.160	0.293

r_{pb}: Point biserial correlation coefficient. P value: Point biserial correlation test.

This table showed no statistically significant correlation between Milan criteria and studied biomarkers (AFP and Midkine).

Table (8): Studied biomarkers in those within and outside Milan criteria

Biomarker	Within Milan	Outside Milan	Z value	P value
AFP(ng/ml)	112 (580)	128 (303)	-0.390	0.697
Midkine(pg/ml)	515 (466)	512.5 (828.5)	-1.226	0.220

Data: Median (IQR). P value: Mann-Whitney U-test.

This table showed no statistically significant difference in AFP and Midkine levels between those within and those outside Milan criteria.

Table (9): Cutoff values to discriminate HCC vs Cirrhosis vs Control

Marker	Cutoff	AUC	SE	95% CI	P value	Sensitivity	Specificity
Cirrhosis vs Control							
AFP(ng/ml)	≥4.1	0.76	0.078	0.60-0.88	<0.001	47%	100%
Midkine(pg/ml)	≥187	0.67	0.098	0.50-0.81	0.090	87%	40%
HCC vs Control							
AFP(ng/ml)	≥4.1	0.96	0.026	0.86-0.99	<0.001	91%	100%
Midkine(pg/ml)	≥485	0.78	0.065	0.65-0.88	<0.001	56%	100%
HCC vs Cirrhosis							
AFP(ng/ml)	≥10	0.90	0.038	0.81-0.96	<0.001	77%	100%
Midkine(pg/ml)	≥508.7	0.68	0.062	0.56-0.78	0.003	56%	87%

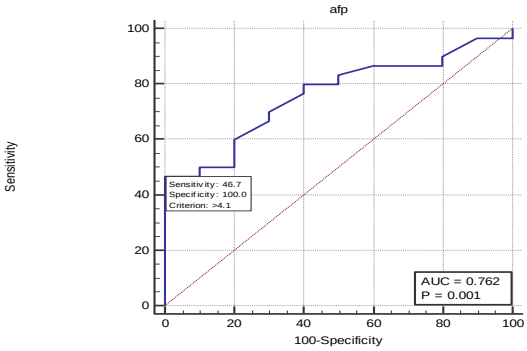

Figure (1): Alpha feto protein to discriminate Cirrhosis from control

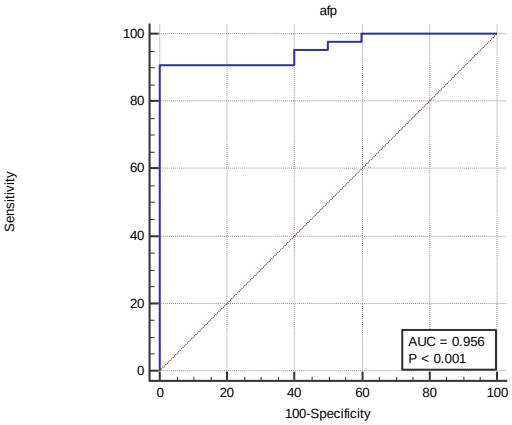

Figure (2): Alpha feto protein to discriminate HCC from control

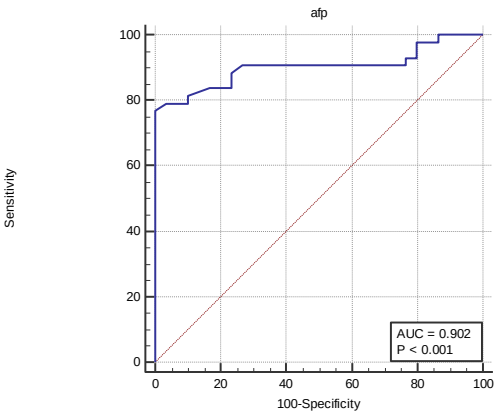

Figure (3): Alpha feto protein to discriminate HCC from cirrhosis

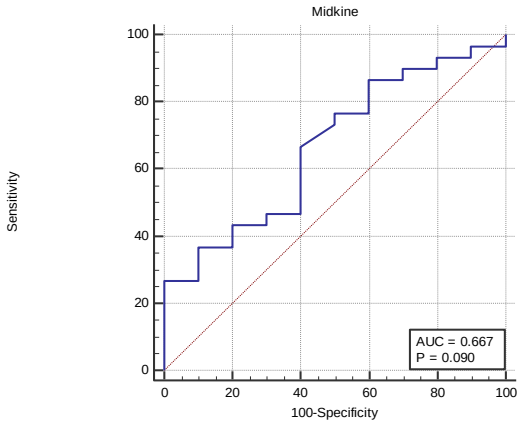

Figure (4): Midkine to discriminate Cirrhosis from control

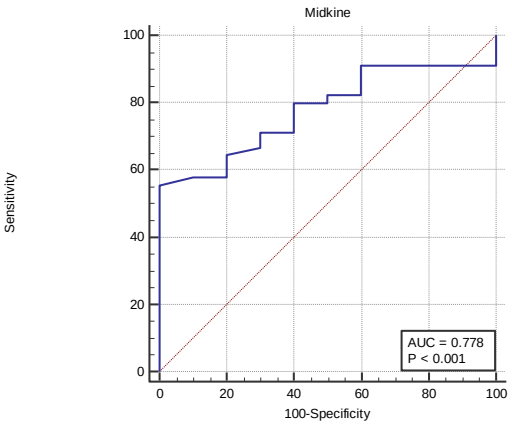

Figure (5): Midkine to discriminate HCC from control

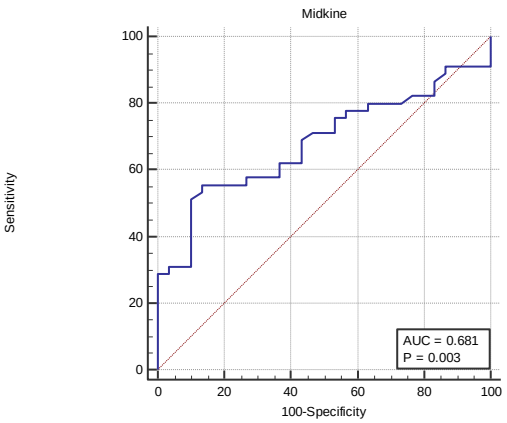

Figure (6): Midkine to discriminate HCC from Cirrhosis

4. DISSCUSION

HCC is the most frequent primary liver malignancy and one of the most malignancies worldwide**. HCC** is considered as the sixth most common cancer type and as the third cause of cancer-related death in the developed countries, more than a million people are dying yearly due to HCC in the Western countries [10].

It was reported that in Egypt, there is rising rate of **HCC** as it has the highest prevalence of **HCV** worldwide [11]**.** At diagnosis the tumor has very often reached an advanced stage and curative treatment options are missing. Thus, early diagnosis would help the patient and prevent increasing healthcare costs [12]**.**

Alpha-fetoprotein(**AFP**) is the most widely used and generally known biomarker for **HCC**, but its use as an independent tool for **HCC** surveillance is not recommended by current guidelines due to its low sensitivity and specificity [13]**.**

Midkine(**MK**) was originally discovered in embryonal carcinoma cells and is involved in the early stage of retinoic acid induced differentiation [14]**.** Midkine also called neurite growth-promoting factor 2 is a plasma secreted protein encoded by the MDK gene on chromosome 11 in humans [15] and is considered a carbohydrate-binding protein. Midkine is overexpressed during tumorigenesis, inflammation and tissue repair [16]**.** Studies have identified Midkine as an **HCC** serum marker and it was identified as one of the five important potential novel biomarkers for early detection of **HCC** [17].

The aim of this study was to evaluate serum Midkine as a marker for hepatocellular carcinoma in HCV-related liver cirrhosis.

The present study included 75 hepatic patients divided into two groups.45 patients with HCC and 30 patients with liver cirrhosis in addition to 10 healthy subjects of matched age and sex as a control group.

Similar study design was reported by [18] in a study to evaluate the clinical significance of serum Midkine levels in the diagnosis of HCC compared with AFP. The study was conducted on 90 patients who were divided into three groups : group I is the HCC group (n=50), group II the liver cirrhosis (n=20), and group III is the control group (n=20).

Also, **Shaheen and his colleagues** conducted a study to evaluate serum midkine as a biomarker for HCC diagnosis. The study included 40 HCC, 30 liver cirrhosis patients, and 30 healthy subjects [19]**.**

In current study, age of HCC group ranged from 49 to 74 years (mean 63.1y $\pm$ 5.5).

This is in agreement with [20] who reported that the most frequent age category affected by **HCC** was between 51 and 60 years.

In this study, the sex distribution revealed a statistically significant difference between the study groups as the majority of the cases in HCC were males (70%) that mean male dominance in HCC group.

This is in agreement with study included total number of 492 patients who were classified into three groups (control,cirrhosis, HCC). The study showed that 74.6% of patients diagnosed with HCC were males [21]**.**

Also, a study by Alves et al. involving 210 patients with HCC reported that 83.3 % of patients were men (76.6 and 83.3%) [22]**.**

El-Edel and his colleagues who revealed that there was as ststistically significant difference between three study groups in their study with predominance of males than females in HCC group (44 males VS 6 females). This sex distribution can be attributed to high prevalence of risk factors like smoking, DM, HCV, and industrial exposure in males in addition to possible role of sex hormones.

In the present study, weight loss was the most common presentation of HCC patients as it presents in 11 cases (24.4%), followed by dyspepsia in 10 cases (22.2%), bleeding in 9 cases (20%), right hypochondrial pain in 6 cases(13.3%) and fatigue in 4 cases(8.8%).

This is in agreement with Ikematsu that weight loss and dyspepsia were the most common presentation (each 28.9%), in HCC and midkine elevation has been reported in 56.5%of patients above the levels observed in the healthy population [23]**.**

Regarding for Biochemical &hematological parameters of the studied groups, we found statistically significant difference between cirrhosis, HCC and control groups with lower platelet and albumin and higher serum bilirubin and INR in HCC group compared to cirrhotic and control group.

This came in contrast with [21 last] who found statistically significant association of HCC with high AST, thrombocytopenia, hypo albuminaemia, increased bilirubin, and prolonged prothrombin time with P less than 0.001 for all parameters.

These findings seem to logic because most of our **HCC** were in early stage often develop in a background of chronic liver disease.

In our study, the median level of **AFP** in the **HCC** group was 116 IU/ml which was higher than its median value in the control and cirrhotic groups with high level of significance between the study groups ($p < 0.001$).

This came in agreement with the well-established data of **AFP** in **HCC** patients. For example, **Yang et al. (2016)** who revealed that the level of serum **AFP** in patients with **HCC** was significantly higher than those of liver cirrhosis and chronic hepatitis patients and healthy controls [24]**.**

The results agreed with those of El Shafie et al.2012 who reported that the serum levels of AFP were significantly elevated in chronic liver diseases and more elevated in HCC cases ($p < 0.001$) **[25].**

In this study, the median level of MK in control group was 238 pg/ml, the median level in cirrhosis group was 292 pg/ml and the median level in HCC group was 538 pg/ml with high level of significance between the different study groups($P<0.002$).

This was in accordance with Shaheen et al. reported that serum MK was significantly elevated in the HCC group compared with cirrhotic and healthy control groups **[26].**

This also agreed with EL-Edel and his colleagues who showed that MK levels were increased in HCC over liver cirrhosis groups; and in HCC over control groups with a highly significant difference. However, there was no significant difference between liver cirrhosis and control groups regarding MK levels (18 last).

The findings that serum midkine is elevated in HCC patients may be clarified by the fact that midkine is an anti apoptotic molecule, as it protects HCC cells from apoptosis mediated by TNF-related apoptosis-inducing ligand (TRAIL) through the reduction of caspase-3 activity **[27] .**

In another study, there was a highly significant statistical difference between the mean value of serum MK levels in patients with HCC compared to patients with liver cirrhosis and the healthy controls ($p<0.001$) **[28].**

In this study, the best cutoff point of midkine to differentiate control subjects from cirrhotic cases was 187 pg/ml with 87% sensitivity, 40% specificity and total accuracy of 64%. Also, the best cutoff point of midkine to differentiate cirrhotic from HCC cases was 508 pg/ml with 56% sensitivity, 87% specificity and total accuracy of 60%.

Mashaly and his colleagues showed that midkine at cut-off value of 1680 pg/ml showed higher sensitivity than that of AFP AT cut-off 200 pg/ml(81.82% versus 52.27%) **[29].**

On the contrary of the previous results, Hung et al. (2011) who found that cut off value of 5000 pg/ml, MK had sensitivity of 51% and specificity of 60% **[30].**

The difference in results can be attributed to difference in the studied population, HCC stages, tumor size, tumor pathology and the number of patients included in both studies. In this study, there was a statistically significant correlation between MK levels with serum AFP levels, tumor size and tumor stage.

In our study among HCC cases in whose AFP was negative, 15 out of 45 patients (33%) , MK level were positive 100%.

These different results reported that a significant limitation to the use of AFP for HCC surveillance is the rate of AFP-negative HCC. Midkine increases the diagnostic yield in AFP-negative HCC and has greated diagnostic performance than AFP (Yang et al., 2019).

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 elevated MK using the optimal diagnostic cut-off of 0.44 ng/ml [31].

In our study, in the group with HCC there were 30 cases with early HCC. Among the cases with early HCC, there were 4 negatives for AFP (8.8%) while the percentage of cases with negative midkine was 0%, including better diagnostic value of MDK in early HCC stages

Zhu et al. (2013) reported by receiver operating characteristic curve analysis showed 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 compared with AFP (80% VS 40%) **[32].**

The results of this study revealed that:

- Serum **AFP** median level was significantly elevated in **HCC** group **(116ng/ml)** when compared with both control **(2.5 ng/ml)** and liver cirrhosis **(3.7 ng/ml)** groups.
- Midkine median level was also significantly elevated in the HCC group (538 ng/I) when compared with both the control (238 ng/I) and liver cirrhosis (292.5 ng/I) groups.
- There were no significant differences were found in MK concentration between males and females in each studied group.
- Also there was no significance association between MK and child pugh classification.
- There was significant difference between the study groups as regards hemoglobin, albumin, creatinine, ALT, AST, platelet count, total bilirubin, and INR but not for WBCs count and direct bilirubin.

Reference

1. Ferlay J., Soerjomataram I., Dikshit R., Eser S., Mathers C., Rebelo M., Parkin D. M., Forman D., and Bray F., Cancer incidence and mortality worldwide : sources, methods and major patterns in Globocan. Int J Cancer, 136(5),E359-386(2015).

2. Atlanta, Aridome K., Tsutsui J., Takao S., Kadomatsu K., Ozawa M., Aikou T., and Muramatsu T., cancer facts and figures American Cancer Society (2014).

3. Jemal A., Bray F., Center M. M., Ferlay J., Ward E., and Forman D., Global cancer statistics. CA Cancer J Clin, 61(2),69-90(2011).

4. Kew M., HCC: epidemiology and risk factors. J Hepatocellular carcinoma, 115-125(2014).

5. El-Houseini, Ismail A, Abdelaal A, EL-Habashy AH, Abdallah and Mohamed M., Role of TGF-B1 and C-Kit mutations in the development of HCC in Hepatitis C-virus infected patients: in vitro study. Biochem(Mosc) (2017).

6. Noha E., Ahmed E. K., Mohamed R. O., Ahmed M. E., Evaluation of Serum Midkine as a Marker of Hepatocellular Carcinoma in Cirrhotic Patients. The Egyptian Journal of Hospital Medicine, 80 (3), 990-996 (2020).

7.Muramatsu H., Zou P., Suzuki H., Oda Y., Chen G. Y., Sakaguchi N., Sakuma S., Maeda N., Noda M., Takada Y., and Muramatsu T., alpha4beta1- and alpha6beta1-integrins are functional receptors for midkine, a heparin-binding growth factor. J Cell Sci,117(pt 22),5405-5415(2004).

8.Mashima T., Sato S., Sugimoto Y., Tsuruo T., and Seimya H., Promotion of glioma cell survival by acyl-CoA synthetase 5 under extracellular acidosis conditions. Oncogene, 28(1),9-19(2009).

9.Hung Y. J., Lin Z. H., Cheng T. I., Liang C. T., Kuo T. M., and Kao K. J., Serum midkine as a prognostic biomarker for patients with HCC. Am J Clin Pathol, 136(4), 594-603 (2011).

10. Dimitroulis, D., Damaskos, C., Valsami, S., Davakis, S., Garmpis, N and Spartalis, E., From diagnosis to treatment of HCC: An epidemic problem for both developed and developing world. World journal of gastroenterology, 23(29),5582(2017).

11. Lehman E. M., and Wilson M. l.,Epidemiology of hepatitis viruses among hepatocellular carcinoma cases and healthy people in Egypt: a systematic review and meta-analysis 23(26),5582 (2009).

12. Plentz, R. and Malek, N., Early detection of HCC: How to screen and follow up patients with liver cirrhosis according to the German S3 guideline?. Diagnostics. 5(4), 497-503(2015).

13.Bruix J., and Sherman M., Management of HCC. Hepatology, 42(5),1208-1236(2005).

14.Weckback, L. T., Muramatsu, T. and Walzog, B., Midkine in inflammation . The Scientific World Journal. 11, 2491-2505 (2011).

15.Kaname T., Kuwano A., Murano I., Uehara K., Muramatsu T., and Kajii T., Midkine gene (MK), a gene for prenatal differentiation and neuroregulation, maps to band 11p11.2 by fluorescence in situ hybridization. Genomics. 17(2), 514-515(1993).

16.Kadomatsu K., and Muramatsu T., Midkine and pleitrophin in neural development and cancer , Cancer Lett . 204(2):127-143(2004).

17.Tsuchiya, N., Sawada, Y., Endo, I., Saito, K., Uemura, Y. and Nakatsura, T., Biomarkers for the early diagnosis of HCC. World journal of gastroenterology: WJG. 21(37), 10573 (2015).

18.El-Edel, R. H., Tawfik, G. A., Noreldin, R. I., El-Jaky, M. A. and Eisaa, D. A., Evaluation of serum Midkine as a novel marker in hepatocellular carcinoma. Menoufia Medical Journal. 31(3),1094(2018).

19.Shaheen K. Y., Abdel-Mageed A. I., Safwat E., and AlBreedy A. M., The value of serum midkine level in diagnosis of HCC. Int J Hepatol. 146389(2015).

20.Shaker, M. K., Abdella, H. M., Khalife, M. O. and El Dorry, A. K., Epidemiological characteristics of hepatocellular carcinoma in Egypt: a retrospective analysis of 1313 cases. Liver Int. 33 (10),1601-1606(2013).

21.El gamal, S., Ghafar, A. A., Ghoneem, E., Elshaer, M., Alrefai, H. and Elemshaty, W., Characterization of patients with hepatocellular carcinoma on the way for early detection: one center experience. The Egyptian Journal of Internal Medicine. 30 (4), 231(2018).

22.Alves, R., Harriz, M., Vanini, H., Alves, D., Soares, S., and Montalveme, T., Carcinoma Hepatocellular: Aspectos Epidemiologicos em 210 Casos. Revista AMRIGS. 53(S3), S43(2009).

23.Ikematsu S., Yano A., Aridome K., Kikuchi M., Kumai K., Nagano H., Okamoto K., Oda M., Sakuma S., Aikou T., Muramatsu H., Kadomatsu K., and Muramatsu T., Serum midkine levels are increased in patients with various types of carcinomas. Br J cancer. 83(6),701-706(2000).

24.Yang, L., Xu, Q., Xie, H., Gu, G. and Jiang., Expression of serum miR-218 in hepatocellular carcinoma and its prognostic significance. Clinical and Translation Oncology.18(8), 841-847 (2016).

25.El Shafie, M. A., Fawzy, A. M., Al Monem, E. A., Abbas, S., Zakaria, D. M. and El Baz, S., Golgi Protein 73 (GP73) as a novel serum marker for early detection of hepatocellular carcinoma in Egyptian patients. Life Sci. J, 9, 823(2012).

26.Shaheen K. Y., Abdel-Mageed A. I., Safwat E., and AlBreedy A. M., The value of serum midkine level in diagnosis of HCC. Int J Hepatol.146389(2015).

27.Ohuchida T., Okamoto K., Akahane K., Higure A., Todoroki H., Abe Y., Kikuchi M., Ikematsu S., Muramatsu T., and Itoh H., Midkine protects HCC cells against TRAIL-mediated apoptosis through down-regulation of caspase-3 activity. Cancer.100(11),2430-2436(2004).

28.Saeed M Youssef N, Sherief AF, Afify S, Wifi M and Omran DGlobal., liver library: an indexed database and Circulating midkine for liver disease. High numbers of Midkine level in peripheral blood of cirrhotic and HCC patients. Clinical Cancer Research. 19(14),3944-3954(2017).

29.Mashaly, A. H., Anwar, R., Ebrahim, M. A., Eissa, L. A. and El Shishtawy, M. M., Diagnostic and prognostic value of Midkine as tumor marker in hepatocellular carcinoma in Egyptian patients. Asian Pacific journal of cancer prevention: APJCP. 19(6),1503(2018).

30.Hung Y. J., Lin Z. H., Cheng T. I., Liang C. T., Kuo T. M., and Kao K. J., Serum midkine as a prognostic biomarker for patients with HCC. Am J Clin Pathol. 136(4), 594-603 (2011).

31.Vongsuvanh, R., Van Der Poorten, D., Iseli, T., Strasser, S. I., McCaughan, G. W. and George,J., Midkine increases diagnostic yield in AFP negative and NASH-related hepatocellular carcinoma. PloS one. 11(5),155-800 (2016).

32.Zhu, W.-W., Guo, J.-J., Guo, L., Jia, H.-l., Zhu, M., and Zhang, J.-B., Evaluation of midkine as a diagnostic serum biomarker in hepatocellular carcinoma . Clinical Cancer Research. 19(14),3944-3954(2013).