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Evaluation of the Role of Serum Midkine (MK) as A New Non-invasive Diagnostic Biomarker for Hepatocellular Carcinoma in Egyptian Cirrhotic Patients

Mohamed Gamal Yousef*, Mohamed Abdelmoghny Mostafa,
Mostafa Osama Mostafa Hashem, Ayman Gamil Anwar
Department of Internal Medicine and Gastroenterology, Faculty of Medicine, Ain Shams University, Egypt
***Corresponding author:** Mohamed Gamal Yousef, **Mobile:** (+20)01003620310,
E-mail: M.gamal.yousef@gmail.com

ABSTRACT

Background: Hepatocellular carcinoma (HCC) is the sixth most prevalent malignancy on a global scale and the second leading cause of cancer-associated mortality in men, following lung cancer.

Objective: This study aimed to assess Midkine (MK) diagnostic utility as an HCC biomarker in Egyptian patients.

Patients and methods: This prospective cross-sectional study was carried out at Ain Shams University Hospitals over six months, involving 80 Egyptian patients with liver cirrhosis (LC): 40 with HCC and 40 without HCC.

Results: The MK levels showed no significant correlation with age, diabetes mellitus (DM), hypertension (HTN), hemoglobin (HB), white blood cell count (WBC), platelet count, aspartate aminotransferase (AST), albumin, or international normalized ratio (INR). However, there were significant positive interactions between MK and alkaline phosphatase (ALP) levels, as well as advanced Barcelona Clinic Liver Cancer (BCLC) stages B and C. Conversely, MK levels were inversely related to Model for End-Stage Liver Disease (MELD) scores. Additionally, MK displayed 87.5% sensitivity (SEN) and 72.5% specificity (SPE) for detecting early-stage HCC, indicating its potential as a diagnostic HCC serum biomarker.

Conclusion: MK may serve as a valuable adjunct to alpha-fetoprotein (AFP) in HCC surveillance programs, particularly for AFP-negative or low-AFP HCC cases. This combination could enhance diagnostic accuracy and early detection in high-risk populations.

Keywords: Serum midkine (MK); Hepatocellular carcinoma; Egyptian cirrhotic patients.

INTRODUCTION

Cancer remains a leading global health challenge, characterized by uncontrolled cellular proliferation and division [(1)]. Hepatocellular carcinoma (HCC), a predominant primary liver cancer subtype, accounts for 70%–85% of incidences and represents a major driver of cancer-related mortality. Globally, HCC is the third most frequent cause of cancer mortalities in 2020 despite being the sixth most commonly diagnosed malignancy [(2)]. In 2023, the USA estimates projected 42,210 new HCC diagnoses, contributing to 4% of cancer-associated mortalities in women and 6% in men [(3)].

Liver cancer ranked among the top three causes of cancer mortality in 46 countries and within the top five in 90 nations. Alarmingly, the annual incidence is projected to surge by 55% between 2020 and 2040 [(4)]. Global epidemiological data highlight the burden of liver cancer, with an incidence rate of 9.3 cases and a mortality rate of 8 deaths per 100,000 person-years [(5)]. Moreover, HCC, which constitutes 70%–85% of all liver cancer diagnoses, is related to a poor prognosis, adversely impacting overall survival and quality of life. Epidemiologically, HCC exhibits marked gender disparities, while men generally face higher risk (male-to-female ratio: 2.4:1), this disparity intensifies among high-risk populations (ratio: 3.7:1) [(6)]. In developed nations, non-cirrhotic HCC cases show near-equal sex distribution. Incidence rises progressively with age across all demographic groups. Despite advancements in surveillance, many HCC cases are diagnosed at advanced stages, limiting therapeutic efficacy and underscoring the urgent need for early diagnostic biomarkers compatible with curative interventions [(7)].

Current HCC surveillance protocols rely on combined radiological imaging and serum biomarkers. Semiannual ultrasound with alpha-fetoprotein (AFP) testing in cirrhotic patients demonstrates 63% sensitivity (SEN) and 84% specificity (SPE) for early detection [(7)]. However, AFP—a widely used biomarker—exhibits suboptimal performance, with about 60% SEN at a 20 ng/mL threshold and poor SPE [(8)]. The AFP remains at normal levels in 15%–30% of advanced HCC cases and may elevate in chronic hepatitis or liver cirrhosis (LC), yielding high false-negative and false-positive rates.

Midkine (MK), a pleiotropic growth factor, emerges as a promising alternative. The MK expression is negligible in healthy adults but significantly upregulated in pathological conditions, including ischemia, inflammation, autoimmunity, and malignancies [(9)].

Notably, MK is detectable in blood and bodily fluids, offering a non-invasive, cost-effective platform for population screening. Preclinical and clinical studies consistently report MK overexpression in various cancers compared to healthy controls, with particular relevance to HCC. These attributes position MK as a promising candidate biomarker for detecting HCC early, potentially complementing existing tools in

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surveillance programs. Accordingly, we aimed to assess MK's diagnostic utility as an HCC biomarker in Egyptian patients.

PATIENTS AND METHODS

This prospective cross-sectional study was conducted at Ain Shams University Hospitals over six months and included 80 Egyptian patients with LC. Participants were equally stratified into the LC group (n = 40), which included patients with LC and no evidence of HCC, and the HCC group, which included patients with LC and confirmed HCC.

Inclusion criteria:

1) Confirmed LC: Patients diagnosed with LC via clinical manifestations, biochemical markers of portal hypertension (HTN), and imaging findings consistent with LC.
2) HCC diagnosis: Patients diagnosed with HCC confirmed using triphasic CT or dynamic contrast-enhanced MRI.

Exclusion criteria:

1) Patients with a previous or current history of other malignancies or autoimmune diseases.
2) Pregnant nursing females.
3) Patients less than 18 years old.
4) Patients refusing to participate.

Study tools: All participants underwent comprehensive clinical, laboratory, and imaging assessments, including a detailed medical history and physical examination, with calculations of Model for End-Stage Liver Disease (MELD) and Child-Pugh scores to assess the severity of liver dysfunction. Laboratory investigations encompassed complete blood count (CBC) with differential liver function tests (Aspartate/alanine aminotransferase [AST]/[ALT], total/direct bilirubin, serum albumin, international normalized ratio [INR]), and renal function tests (Creatinine, blood urea nitrogen [BUN], sodium & potassium), alongside tumor markers AFP and MK. Imaging studies included abdominal ultrasound as well as tri-phase CT or dynamic MRI, which were analyzed for lesion count, size, location, portal vein thrombosis, vascular invasion, and metastatic spread.

Ethical approval: This study was approved by Ain shams Faculty of Medicine's Ethics Committee [No.: FWA000017585]. Following receipt of all information, signed consent was provided by each participant. The study adhered to the Helsinki Declaration throughout its execution.

Statistical analysis

Data analyses were conducted using SPSS version 27.0. Quantitative variables were reported as means, standard deviations, and ranges for parametric data, while medians and interquartile ranges (IQR) were reported for non-parametric distributions. Qualitative variables were described as frequencies and percentages. Statistical significance was determined by p-values: non-significant (NS) for $p > 0.05$, significant (S) for $p \leq 0.05$, and highly significant (HS) for $p \leq 0.01$.

RESULTS

Table (1) demonstrated no significant differences in age ($p = 0.320$), gender ($p = 0.116$), prevalence of diabetes mellitus (DM) ($p = 0.651$), HTN ($p = 0.799$), or smoking status ($p = 0.356$) between the two groups.

Table (1): Demographic and clinicopathological comparisons between groups

		HCC	LC	Test value	P-value	Sig.
		No. = 40	No. = 40			
Age	Mean ± SD Range	52.8 ± 12.63 31–76	55.53± 11.72 30–80	–1.000•	0.320	NS
Gender	Female Male	15 (37.5%) 25 (62.5%)	22 (55.0%) 18 (45.0%)	2.464*	0.116	NS
DM	Negative Positive	24 (60.0%) 16 (40.0%)	22 (55.0%) 18 (45.0%)	0.205*	0.651	NS
HTN	Negative Positive	29 (72.5%) 11 (27.5%)	30 (75.0%) 10 (25.0%)	0.065*	0.799	NS
Smoking	Negative Positive	23 (57.5%) 17 (42.5%)	27 (67.5%) 13 (32.5%)	0.853*	0.356	NS

*Chi-square test; •Independent t-test

In LC patients, hemoglobin (Hb) levels were significantly higher (p = 0.041), while total bilirubin levels were significantly lower (p = 0.018) compared to HCC patients. Alkaline phosphatase (ALP) (p = 0.017) and gamma-glutamyl transferase (GGT) levels (p = 0.043) significantly differed between the groups. Additionally, potassium levels exhibited a significant difference (p = 0.022, **Table 2**).

Table (2): Laboratory parameter differences between groups

		HCC	LC	Test value	P-value	Sig.
		No. = 40	No. = 40			
TLC	Median (IQR) Range	7.55 (5.2–10.85) 2.2–22	5.5 (4.2–8) 2.2–22	–1.959≠	0.050	NS
Hb (g/dL)	Mean ± SD	9.13±1.5	9.9±1.79	–2.075•	0.041	S
PLT (mcL)	Mean ± SD	105.53±25.78	107.7±26.81	–0.225•	0.823	NS
INR	Mean ± SD	1.9±0.46	2.01±0.49	–0.664•	0.509	NS
AST (U/L)	Median (IQR) Range	34 (29.5–55) 11–88	32.5 (24–41.5) 18–200	–1.060≠	0.289	NS
ALT (U/L)	Median (IQR) Range	46.5 (40–67) 26–99	47 (38.5–58.5) 27–221	–0.274≠	0.784	NS
Albumin (g/dL)	Mean ± SD	2.84±0.43	2.94±0.44	–1.053•	0.296	NS
Total bilirubin (µmol/L)	Mean ± SD	2.91±0.71	2.44±0.60	2.425•	0.018	S
Direct bilirubin (µmol/L)	Median (IQR) Range	1 (0.7–1.3) 0.4–2.6	0.8 (0.6–1.05) 0.4–9	–1.503≠	0.133	NS
ALP (U/L)	Median (IQR) Range	97.5 (69–137) 48–300	76 (57.5–87.5) 39–287	–2.397≠	0.017	S
GGT (U/L)	Median (IQR) Range	54 (34–72.5) 1–123	38.5 (32–54) 17–113	–2.020≠	0.043	S
BUN (mg/dL)	Mean ± SD	19.2±4.68	22.35±5.52	–1.827•	0.071	NS
Creat (mg/dl)	Mean ± SD	0.97±0.23	1.12±0.27	–1.392•	0.168	NS
Na (mmol/L)	Mean ± SD	128.42±4.15	128.37±3.36	0.059•	0.953	NS
K (mmol/L)	Mean ± SD	3.95±0.71	3.61±0.62	2.336•	0.022	S

Median, IQR and range: Non-para mtric test. •Independent t-test; ≠: Mann-Whitney test

Table (3) indicated a significant difference in hepatitis C virus antibodies (HCV Ab) prevalence between groups, with a higher proportion observed in HCC patients, unlike LC patients (p = 0.039).

Table (3): Comparisons of viral markers in both groups.

		HCC	LC	Test value	P-value	Sig.
		No. = 40	No. = 40			
HbsAg	Negative Positive	37 (92.5%) 3 (7.5%)	36 (90.0%) 4 (10.0%)	0.157*	0.692	NS
HCV Ab	Negative Positive	6 (15.0%) 34 (85.0%)	14 (35.0%) 26 (65.0%)	4.267*	0.039	S

*Chi-square test.

The HCC patients displayed significantly higher MK levels than the LC patients (P < 0.001). Additionally, AFP levels were significantly heightened in HCC patients relative to LC patients (p < 0.001) (Table 4).

Table (4): Comparative analysis of AFP and MK levels in both groups

		HCC	LC	Test value	P-value	Sig.
		No. = 40	No. = 40			
AFP	Median (IQR) Range	44.15 (11.3–800.5) 1.6–44876	2.6 (1.8–7.3) 0.7–43.4	–5.530≠	< 0.001	HS
MK	Median (IQR) Range	771.55 (458.25–2225) 143.8–2400	321.9 (238.75–433.5) 169.8–2400	–5.312≠	< 0.001	HS

Median, IQR and Range: Non-paramtric test. ≠: Mann-Whitney test

There were no significant disparities between both groups in Child class/score and MELD score (P = 0.820, 0.323, and 0.497 respectively, **Table 5**).

Table (5): Severity scores (Child-Pugh and MELD) in both groups

		HCC	LC	Test value	P-value	Sig.
		No. = 40	No. = 40			
Child	B	24 (60.0%)	23 (57.5%)	0.052*	0.820	NS
	C	16 (40.0%)	17 (42.5%)			
Child	Mean ± SD	9.5±1.83	9.08±1.99	0.995•	0.323	NS
	Range	7–14	6–13			
MELD	Mean ± SD	18.1±3.79	18.75±4.68	-0.683•	0.497	NS
	Range	11–26	10–31			

*: Chi-square test; •: Independent t-test

Table (6) showed no significant correlation between MK level and the other parameters among LC patients.

Table (6): Correlation of MK with laboratory parameters in LC patients

LC group	MK	
	R	P-value
Age (years)	0.176	0.278
TLC	–0.117	0.472
HB (g/dL)	0.148	0.361
PLT (mcL)	–0.095	0.559
INR	0.039	0.813
AST (U/L)	–0.061	0.707
ALT (U/L)	–0.077	0.636
Albumin (g/dL)	–0.205	0.204
Total bilirubin (µmol/L)	0.109	0.504
Direct bilirubin (µmol/L)	0.118	0.467
ALP (U/L)	–0.106	0.516
GGT (U/L)	–0.044	0.789
BUN (mg/dL)	–0.119	0.463
Creat (mg/dl)	–0.118	0.467
Na (mmol/L)	–0.089	0.585
K (mmol/L)	–0.147	0.364
AFP	0.010	0.950
Child	0.175	0.279
MELD	0.043	0.794

Spearman correlation coefficient

The results revealed that MK levels exhibited a significant negative correlation with MELD scores in HCC patients (p = 0.022). Meanwhile, AFP levels showed no significant associations (Table 7).

Table (7): Association of AFP and MK with liver disease severity scores in HCC patients

HCC	AFP		MK	
	r	P-value	r	P-value
Child	**–0.060**	**0.713**	**-0.214**	**0.185**
MELD	**0.015**	**0.929**	**-0.362***	**0.022**

Spearman correlation coefficient.

Table (8) showed that MK levels were significantly associated with Barcelona Clinic Liver Cancer (BCLC) stages B/C compared to stages A/D (p = 0.010).

Table (8): Clinical determinants of AFP and MK Levels in HCC patients

HCC		AFP	Test value	P-value	Sig.	MK	Test value	P-value	Sig.
		Median (IQR)				Median (IQR)			
HBsAg	Negative	46.8 (14.7–935)	–0.796•	0.426	NS	956.5 (487.4–2227)	–1.678•	0.093	NS
	Positive	33 (4.9–44.3)				376.3 (321.3–768.7)			
HCV Ab	Negative	355.15 (17.2–6516.8)	–0.663•	0.507	NS	1908 (768.7–2400)	–1.200•	0.230	NS
	Positive	38.5 (7.9–419)				656.35 (453.7–2223)			
Child	B	45.4 (11.3–800.5)	–0.138•	0.890	NS	1329 (554.3–2225)	–1.833•	0.067	NS
	C	38.65 (11.05–595.5)				465.55 (348.8–1981)			
BCLC stage	A	33 (10.05–161.5)	3.374≠	0.338	NS	585.4 (530.35–1336.5)	11.346≠	0.010	S
	B	26.14 (7.9–666)				1422 (534.7–2083)			
	C	2000 (419–10220)				2400 (2400–2400)			
	D	38.65 (11.05–595.5)				465.55 (348.8–1981)			

•: Mann-Whitney test; ≠: Kruskal-Wallis test

Table (9) showed a significant positive correlation between MK and ALP levels and a negative correlation between MK levels and MELD score, with no correlation between MK levels and the other parameters among HCC patients.

Table (9): Correlation of MK with laboratory parameters in HCC patients

HCC group	MK	
	r	P-value
Age (years)	0.095	0.560
TLC	0.282	0.077
HB (g/dL)	–0.075	0.645
PLT (mcL)	0.242	0.132
INR	–0.250	0.119
AST (U/L)	0.000	0.998
ALT (U/L)	0.016	0.920
Albumin (g/dL)	0.119	0.464
Total bilirubin (µmol/L)	–0.218	0.176
Direct bilirubin (µmol/L)	–0.221	0.171
ALP (U/L)	**0.383***	**0.015**
GGT (U/L)	0.232	0.149
BUN (mg/dL)	–0.211	0.192
Creat (mg/dl)	0.005	0.973
Na (mmol/L)	0.122	0.451
K (mmol/L)	–0.309	0.053
AFP	0.276	0.085
Child	–0.214	0.185
MELD	**–0.362***	**0.022**

Spearman correlation coefficient

Table (10) showed a significant increase in the level of MK in patients at BCLC stages B/C than in patients at BCLC stages A/D (P = 0.010).

Table (10): Clinical associations of MK in HCC patients

HCC group		MK		Test value	P-value	Sig.
		Median (IQR)	Range			
Gender	Female	956.5 (443.7–1562)	302.6–2400	–0.506•	0.619	NS
	Male	716.9 (487.4–2400)	143.8–2400			
DM	Negative	572.65 (458.25–2225)	302.6–2400	–1.111•	0.267	NS
	Positive	1499.5 (519.75–2241.5)	143.8–2400			
HTN	Negative	768.7 (487.4–2223)	302.6–2400	–0.137•	0.891	NS
	Positive	956.5 (422–2400)	143.8–2400			
Smoking	Negative	768.7 (422–2223)	302.6–2400	–0.908•	0.364	NS
	Positive	956.5 (538.3–2400)	143.8–2400			
HbsAg	Negative	956.5 (487.4–2227)	143.8–2400	–1.678•	0.093	NS
	Positive	376.3 (321.3–768.7)	321.3–768.7			
HCV Ab	Negative	1908 (768.7–2400)	422–2400	–1.200•	0.230	NS
	Positive	656.35 (453.7–2223)	143.8–2400			
Child	B	1329 (554.3–2225)	422–2400	–1.833•	0.067	NS
	C	465.55 (348.8–1981)	143.8–2400			
BCLC stage	A	585.4 (530.35–1336.5)	422–2223	11.346≠	0.010	S
	B	1422 (534.7–2083)	462.8–2227			
	C	2400 (2400–2400)	2400–2400			
	D	465.55 (348.8–1981)	143.8–2400			

•Mann-Whitney test; ≠: Kruskal-Wallis test.

The ROC curve analysis demonstrated the AFP diagnostic performance and MK in differentiating HCC from LC. For AFP, the optimal cut-off value was > 3.4, yielding 92.5% SEN and 65.0% SPE, with an AUC of 0.859. MK exhibited an optimal cut-off of > 388.2, achieving 87.5% SEN and 72.5% SPE, with an AUC of 0.845. Both biomarkers showed strong discriminatory power, with AUC values exceeding 0.8, indicating high diagnostic accuracy.

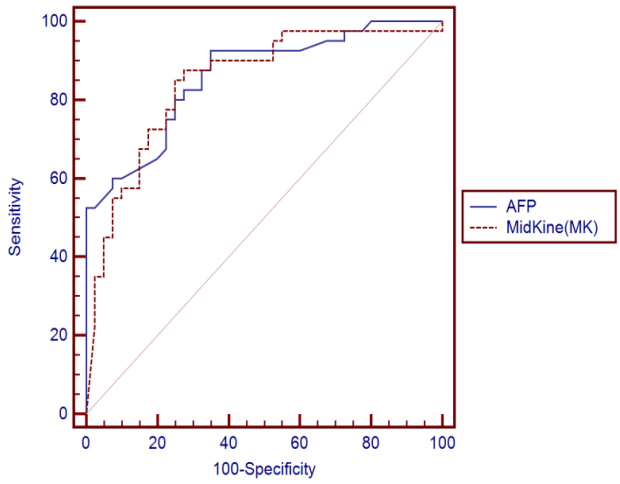

Figure (1): ROC curve evaluation of AUC, SEN, and SPE of MK and AFP as diagnostic biomarkers for HCC and LC patients.

DISCUSSION

Globally, HCC is the sixth most prevalent malignancy and the fourth most common cancer in Egypt [(10)]. Late diagnosis remains a critical challenge, limiting therapeutic efficacy and underscoring the urgent need for novel biomarkers to enable early detection and monitoring. MK, a 13 kDa cysteine-rich protein encoded by the MDK gene on chromosome 11 [(11)], has emerged as a promising candidate. Besides being termed neurite growth-promoting factor-2 (NEGF-2) or retinoic acid-inducible factor, MK is minimally expressed in normal adult tissues but highly upregulated during embryogenesis and pathological conditions, including cancer [(12)].

This study aimed to evaluate MK's diagnostic utility for HCC, focusing on its potential to improve early detection and clinical outcomes. Herein, the median age of HCC patients (52.8±12.63 years) aligns with epidemiological trends indicating peak incidence in the fifth to sixth decades [(13)], consistent with prior observations in Egyptian populations. Our results showed no significant disparity between the HCC and LC groups in the percentage of patients with DM, HTN, and smoking (p = 0.651, 0.799, and 0.356, respectively), indicating that smoking is not HCC risk factor. This disagreed with **Trichopoulos *et al.*** [(14)] who reported that smoking might be HCC risk factor (47.6% of HCC was associated with smoking).

Although the relationship between HTN and HCC is not clearly established in our study, **Hu *et al.*** [(15)] suggested that HTN is HCC risk factor and is linked to poor prognosis. Contrary to our findings about the association with DM, several observational studies from North America, Asia, and Europe, and later meta-analyses, support the notion that insulin resistance and DM are separate risk factors for HCC [(4)], possibly because of the limitations of the study sample.

This study demonstrated significantly that heightened AFP levels in HCC patients, unlike LC patients (p < 0.001), corroborates findings by **Omran *et***

al. [16]. In their multicenter study involving 196 patients (104 HCC, 52 LC, 40 liver fibrosis) and a validation cohort of 122 patients (80 HCC, 42 LC), AFP exhibited an AUC of 0.69 at a 400 IU/mL cut-off, yielding 29% SEN (30/104 HCC patients) but high SPE. In contrast, our analysis identified a lower optimal AFP cut-off (> 3.4 IU/mL), achieving 92.5% SEN and 65.0% SPE for distinguishing HCC from LC, emphasizing its utility in early detection despite reduced SPE. Meanwhile, the MK level displayed a significant elevation in HCC patients than in LC patients (P <0.001), which agrees with **Mashaly *et al.*** [17]. Their study of 75 participants (44 HCC, 31 LC, 15 healthy controls) reported MK elevation in HCC relative to LC and controls ($p < 0.001$), validating its diagnostic potential.

Omran *et al.* [16] assessed MK serum levels in two cohorts: 104 patients having HCC and 92 having non-malignant liver disease, followed by a validation cohort of 80 HCC and 42 LC patients. Their findings demonstrated significantly higher MK levels in HCC patients than in those with LC, supporting its potential as a diagnostic biomarker for distinguishing HCC from non-malignant hepatic conditions. Similarly, **Malov *et al.*** [18] assessed MK levels in 55 patients with chronic HCV-related LC without HCC and 55 with HCV-related LC and concurrent HCC. This study also identified MK as a robust diagnostic marker, exhibiting high SEN for HCC detection even in the presence of cirrhosis. Together, these studies underscore MK's utility in enhancing the accuracy of HCC diagnosis, particularly in differentiating malignant from non-malignant liver pathology.

This study corroborates prior research in Egyptian populations, validating MK as a biomarker for HCC, including **Elnakeeb *et al.*** [19]. Their cohort of 90 participants was stratified into group I (n=40, HCC with LC), group II (n=40, HCV-related LC without HCC), and group III (n=10, healthy controls). **Elnakeeb *et al.*** [19] reported significantly elevated MK levels in HCC patients in contrast to both LC and controls ($p < 0.001$), with MK concentrations increasing alongside tumor size and multiplicity. ROC analysis identified an optimal MK cut-off of 8500 pg/mL, achieving 100% SEN and 87.5% SPE for distinguishing HCC from LC, with 94.5% diagnostic accuracy**.** In contrast, our study demonstrated superior biomarker performance at a lower MK cut-off of >388.2 pg/mL, yielding 87.5% SEN and 72.5% SPE. These findings align with the broader evidence base, underscoring MK's diagnostic utility in HCC detection, particularly in populations with high HCV prevalence.

AbdElaleem *et al.* [2] reported comparable findings supporting MK as a superior diagnostic biomarker to AFP for HCC. Their study stratified participants into three cohorts: group I (n=30, HCC on HCV), group II (n=30, HCV-related LC), and a control group (n=30, healthy adults). The MK demonstrated robust discriminatory power between HCC and LC at a cut-off of > 97.7 pg/mL, achieving 80% SEN and 90% SPE. In this study, multivariate logistic regression analysis identified MK > 388.2 pg/mL as the strongest independent predictor of HCC (OR: 105.88, 95% CI: 5.73–1956.6; $p = 0.002$), followed by AFP > 3.4 IU/mL (OR: 72.19, 95% CI: 5.02–1038.5). These results align with prior evidence validating MK's diagnostic superiority over AFP, particularly in populations with HCV-related liver disease.

Patients at BCLC stages B/C had significantly higher MK levels than patients at BCLC stages A/D ($P = 0.010$), which is consistent with **Darmadi *et al.*** [3], wherein 100 HCC patients showed higher MK in tumor sizes > 5 cm than those with sizes < 3 cm, which aligns with **Elnakeeb *et al.*** [19]. However, these results contrast with **Omar *et al.*** [20], who elucidated no significant correlations between MK levels and BCLC stage, tumor size, or number in 90 participants (40 HCV-related HCC, 40 LC, 10 controls). Discrepancies may arise from differences in sample size, disease heterogeneity, or assay methodologies, underscoring the need for standardized validation across diverse populations.

This study found no significant correlations between MK levels and Child-Pugh score, MELD score, or Child classification ($p > 0.05$), which is consistent with **Omar *et al.*** [20] who reported no discernible associations between MK and these prognostic scores in HCC. However, a significant positive correlation emerged between MK and ALP levels, which is aligning with **Yu *et al.*** [21] who observed hierarchical clustering of ALP (> 82 IU/L) in 1,685 HCC cases, suggesting MK's potential role in biliary dysfunction or tumor-associated metabolic dysregulation.

In our study, ROC analysis identified an optimal MK cut-off of > 388.2 pg/mL for distinguishing HCC from LC, achieving 87.5% SEN and 72.5% SPE (AUC: 0.845). In comparison, AFP demonstrated lower SPE (65.0%) but superior SEN (92.5%) at a cut-off of >3.4 IU/mL (AUC: 0.859). While MK exhibited higher SEN than AFP, its SPE variability across studies raises questions about its consistency as a standalone biomarker. These findings underscore MK's complementary value to AFP, particularly in detecting low-AFP HCC subtypes, though standardization of cut-offs and integration with imaging or clinical scores may enhance diagnostic reliability.

CONCLUSION

The integration of MK into HCC surveillance programs holds significant promise for improving diagnostic accuracy, particularly when combined with AFP. This approach is especially critical for detecting HCC in patients with low AFP levels, where standalone AFP testing may lack SEN.

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ABBREVIATIONS

AFP	Alpha-Fetoprotein
ALT	Alanine Aminotransferase
ALP	Alkaline Phosphatase
AST	Aspartate Aminotransferase
AUC	Area Under the Curve (ROC Analysis)
BCLC	Barcelona Clinic Liver Cancer Staging System
BUN	Blood Urea Nitrogen
Child	Child-Pugh Score (Liver Dysfunction Severity)
Creat	Creatinine
DM	Diabetes Mellitus
GGT	Gamma-Glutamyl Transferase
HB	Hemoglobin
HBsAg	Hepatitis B Surface Antigen
HCC	Hepatocellular Carcinoma
HCV Ab	Hepatitis C Virus Antibodies
HTN	Hypertension
HS	Highly Significant ($P < 0.01$)
INR	International Normalized Ratio
IQR	Interquartile Range
K	Potassium
LC	Liver Cirrhosis
MELD	Model for End-Stage Liver Disease
MK	Midkine
Na	Sodium
NS	Non-Significant ($P > 0.05$)
PL	Platelet Count
r	Spearman Correlation Coefficient
S	Significant ($P < 0.05$)
SD	Standard Deviation
TLC	Total Leukocyte Count

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