Evaluation of serum dickkopf-1 as a novel biomarker for hepatocellular carcinoma

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Background
Hepatocellular carcinoma (HCC) is a common worldwide cancer. α-Fetoprotein (AFP) is a routinely used biomarker for HCC diagnosis, but with reduced clinical applicability due to low sensitivity and specificity. Dickkopf-1 (DKK-1) is vital in the differentiation, survival, apoptosis, and cell death. DKK-1 has a potential oncogenic role in carcinogenesis.

Aim
In this study, we evaluated the diagnostic and prognostic performance of serum DKK-1, AFP, and their combination in HCC.

Patient and methods
This study was done on 40 HCC patients, 24 liver cirrhosis patients, and 16 age-matched and sex-matched healthy controls. The patients were selected from the Tropical Medicine and Gastroenterology Department, Al-Rajhi Liver Hospital.

Results
The optimum cutoff for DKK-1 in HCC patients versus liver cirrhosis and control groups was more than 331 pg/ml with a sensitivity of 80.0% and specificity of 87.5%. The optimum cutoff for AFP was more than 8 IU/ml (sensitivity 77.5%, specificity 85.0%).The combination of the two markers had the best sensitivity (92.5%) in the diagnosis of HCC patients.

Conclusion
DKK-1 levels was significantly higher in newly diagnosed HCC patients than in the nonmalignant control group. The combination of the two markers (DKK-1 and AFP) enhanced the sensitivity.

Keywords:
α-fetoprotein, dickkopf-1, hepatocellular carcinoma

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Introduction

Hepatocellular carcinoma (HCC) is the most common type of liver cancer. Globally, it is the sixth most common cancer and the second cause that leads to mortality from tumors [1]. In Egypt, HCC is the fourth most frequent cancer and is the second cause of cancer death in men and women [2].

National Comprehensive Cancer Network (2012) guidelines recommended serum α-fetoprotein (AFP) measurement and ultrasound every 6–12 months as a screening strategy for HCC in high-risk patients [3].

AFP is the current marker for differentiating HCC from cirrhosis with no HCC. However, serum AFP is associated with two main problems: (a) low specificity as a transient rise in the serum level of AFP could occur during exacerbation of chronic hepatitis, acute hepatitis, and liver cirrhosis (LC). (b) Low sensitivity as AFP levels may be normal in 40% of HCC patients. So, false positive and negative results could occur [4]. Abdominal ultrasound is dependent on the examiner's experience and cannot discriminate between malignant and benign nodules [5].

Therefore, there is need for novel serum biomarkers with higher sensitivity and specificity for early HCC diagnosis [6].

Dickkopf-1 (DKK-1) is a protein involved in head formation in embryonic development. Several studies demonstrated that DKK-1 had a role in the control of different pathological and physiological processes, including adult hippocampal neurogenesis [7], osteoclastogenesis [8], proliferation of tumor cells, migration, invasion, and survival [9].

DKK-1 has an elevated expression in the serum of patients with HCC. Qi *et al.* [10] reported that HCC patients had a higher serum DKK-1 level compared with the controls and non-HCC liver disease patients.

Aim

The aim of this study was to evaluate the diagnostic performance of serum DKK-1, AFP, and their combination in HCC and to determine the prognostic value of serum DKK-1 in HCC by studying its correlation with the Barcelona Clinic Liver Cancer (BCLC) staging system.

Patients and methods

This study was performed on 40 HCC patients, 24 LC patients, and 16 age-matched and sex-matched healthy controls. The patients were selected from the Tropical Medicine and Gastroenterology Department, Al-Rajhi Liver Hospital, Assiut University over a period of 1 year duration from March 2017 to March 2018.

Classification of patients

(1) HCC group: 40 patients and they were classified according to BCLC staging as given below.
   (a) Stage 0 (very early stage) HCC: 10 patients.
   (b) Stage A (early stage) HCC: 10 patients.
   (c) Stage B (intermediate stage) HCC: 10 patients.
   (d) Stage C and D (late stage) HCC: 10 patients.

According to Child–Pugh score, HCC patients were divided as follows.
   (i) Class A: 27 patients.
   (ii) Class B: eight patients.
   (iii) Class C: five patients.

(2) LC group: 24 patients.

They were classified according to the Child–Pugh score as in the following.
   (a) Class A: eight patients.
   (b) Class B: eight patients.
   (c) Class C: eight patients.

(3) Control group: 16 apparently healthy personnel, sex-matched and age-matched with both patient groups.

Sample collection, storage, and handling

Random blood sample: a volume of 8 ml of venous blood was withdrawn under complete aseptic conditions and were divided into:

(1) A volume of 2 ml was collected into an EDTA containing tube for blood count.
(2) A volume of 2 ml was collected into a sodium citrate containing tube for prothrombin time and concentration.
(3) A volume of 4 ml was collected into a plain tube without anticoagulants, centrifuged at a speed of 2000–3000 rpm for 20 min and stored at −80°C for kidney functions, liver functions, AFP, and assay of human DKK-1 level.

Routine investigations

Serum urea, serum creatinine, and liver functions were done on COBAS Integra 400 plus (Roche, Mannheim, Germany). Prothrombin time and concentration were done on a Sysmex CA-1500 System (Siemens, Munich, Germany). Complete blood count was done on ABX Pentra XL 80 (HORIBA Medical, Montpellier, France).

Special investigations

Serum AFP was done on MAGLUMI fully autochemiluminescence immunoassay analyzer (MAGLUMI 2000, Shenzhen New Industries Biomedical Engineering Co., Ltd., Shenzhen, China).

Serum DKK-1 level determination: was measured by enzyme-linked immunosorbent assay technique using human DKK-1 ELISA Kit (Catalog No: SG-10783; SinoGeneClon Biotech Co., Hangzhou, China).

Principle of the test

Enzyme-linked immunosorbent assay was based on the sandwich immunoassay principle. The assay uses two highly specific monoclonal antibodies for the detection of tested antigen; one antibody is immobilized into the microplate and the other one is labeled to form a sandwich complex (antibody–antigen-labeled antibody). Absorbance is measured spectrophotometrically at 450 nm.

Statistical analysis

Data entry and data analysis were done using the statistical package for social sciences, version 19. Data were presented as mean, SD/SEM and median. Mann–Whitney test was used to compare the quantitative variables between groups and in case of nonparametric data. MedCalc was used to calculate the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) and receiver operating characteristic curves. *P* value is considered statistically significant when the *P* value is less than 0.05.

Ethical consideration

Formal consent was obtained from patients and controls. The study was approved by the Ethics Committee of Faculty of Medicine, Assiut University.

Results

Serum levels of dickkopf-1 and α-fetoprotein in patients and control groups

HCC group had significantly higher DKK-1 when compared with control and LC groups (P < 0.001 for both), but no significant difference could be detected between the LC and control groups (P = 0.384) (Table 1).

HCC group had significantly higher AFP in comparison with the control and LC groups (P < 0.001 for both), but no significant difference could be detected between the LC and control groups (P = 0.679).

Serum levels of dickkopf-1 and α-fetoprotein in early-stage hepatocellular carcinoma (stages 0 and A) and intermediate-stage and late-stage hepatocellular carcinoma (stages B, C, and D)

DDK-1 mean value showed significant increase in stages B+C+D than stages 0+A (P = 0.006). Comparison between these two groups regarding the AFP mean value showed no significant difference between them (P = 0.350) (Table 2).

Study of the diagnostic performance of dickkopf-1 and α-fetoprotein in hepatocellular carcinoma

Diagnostic performance of dickkopf-1, α-fetoprotein, and their combination for distinguishing hepatocellular carcinoma from the nonmalignant group (liver cirrhosis and control groups)

The optimum cutoff for DKK-1 in HCC patients versus LC and control groups was more than 331 pg/ml with an area under the curve (AUC) of 0.847, sensitivity of 80.0%, and specificity of 87.5%. The optimum cutoff for AFP was more than 8 IU/ml (AUC 0.830, sensitivity 77.5%, specificity 85.0%). There was no significant difference between the two markers in AUC (P = 0.796). When using the currently recommended clinical cutoff for AFP (20 ng/ml), the sensitivity was 50.0% and the specificity was 97.0% with an AUC 0.738 (Table 3, Figs. 1 and 2).

The combination of tumor markers in HCC patients versus LC and control groups was considered positive if any one of the markers' level was above the cutoff value (AFP >8 IU/ml or DKK-1 >331 pg/ml). In contrast, the combination of tumor markers was considered negative only if all the markers were below the cutoff value (AFP < 8 IU/ml and DKK-1 <331 pg/ml). In the ROC analysis, combining the two tumor markers in HCC patients versus LC and control groups had the best sensitivity (92.5%) in the diagnosis of HCC patients. There was no significant difference between the combination of DKK-1 and AFP compared with the use of a single marker either DKK-1 or AFP in AUC (P = 0.949 and 0.955, respectively).

Also, the combination of the two tumor markers in early-stage HCC patients (BCLC stages 0 and A) versus LC and control groups had the best sensitivity (85.0%) in the diagnosis of early-stage HCC patients. There was no significant difference between the combination of DKK-1 and AFP compared with the use of a single marker either DKK-1 or AFP in AUC (P = 0.927 and 0.882, respectively).

Diagnostic performance of dickkopf-1, α-fetoprotein, and their combination for distinguishing hepatocellular carcinoma from high-risk patients (liver cirrhosis group)

In this study, we classified LC as patients at high risk. We determined the role of both markers in

Table 1 Serum levels of dickkopf-1 and α-fetoprotein in patients and control group

	HCC (n=40)	LC (n=24)	Control (n=16)	P^a	P^b	P^c
Dickkopf-1 (pg/ml)						
Mean±SD	407.63±135.75	284.19±59.41	280.38±37.37	<0.001*	<0.001*	0.384 (NS)
Range	174-682.5	133.5-372.5	229-354			
AFP (IU/ml)						
Median (IQR)	30.6 (8.48-532.88)	5.1 (2.88-7.53)	4.6 (3.48-6.23)	<0.001*	<0.001*	0.679 (NS)

AFP, α-fetoprotein; HCC, hepatocellular carcinoma; IQR, interquartile range; LC, liver cirrhosis. [a]P: comparison between HCC and LC. [b]P: comparison between HCC and control. [c]P: comparison between LC and control. P>0.05, NS. *P<0.05, statistically significant difference.

Table 2 Serum level of sickkopf-1 and α-fetoprotein in early-stage hepatocellular carcinoma (stages 0 and A) and intermediate-stage and late-stage hepatocellular carcinoma (stages B, C, and D)

	BCLC		P
	Stages 0+A (n=20)	Stages B+C+D (n=20)	
DKK-1 (pg/ml)			
Mean±SE	345.30±15.70	469.95±35.15	0.006*
Median (range)	351.8 (174.0-484.0)	397.5 (190.0-682.5)	
AFP (IU/ml)			
Median (IQR)	22.1 (22.05-370.48)	40.0 (9.40-897.50)	0.350 (NS)

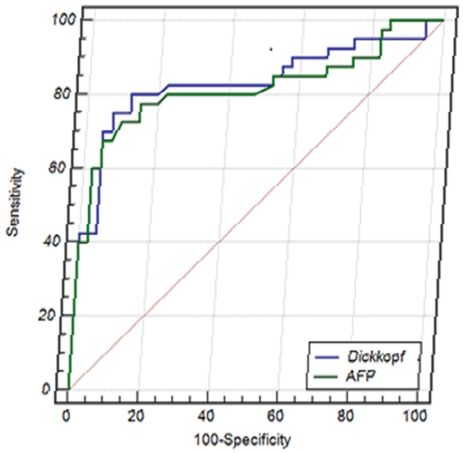
AFP, α-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; DKK-1, dickkopf-1; IQR, interquartile range.

Table 3 Performance of α-fetoprotein, dickkopf-1, and their combination for the diagnosis of hepatocellular carcinoma and early-stage hepatocellular carcinoma versus liver cirrhosis and control groups

	Cutoff	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	AUC
HCC versus LC and control groups							
DKK-1	>331	80.0	87.5	86.5	81.4	83.8	0.847
AFP	>8	77.5	85.0	83.8	79.1	81.3	0.830
DKK-1 and AFP	AFP>8 or DKK-1 >331	92.5	75.0	78.7	90.9	83.8	0.838
Early-stage HCC versus LC and control groups							
DKK-1	>331	70.0	87.5	73.7	85.4	81.7	0.787
AFP	>8	75.0	85.0	71.4	87.2	81.7	0.821
DKK-1 and AFP	AFP >8 or DKK-1 >331	85.0	75.0	63.0	90.9	78.3	0.800

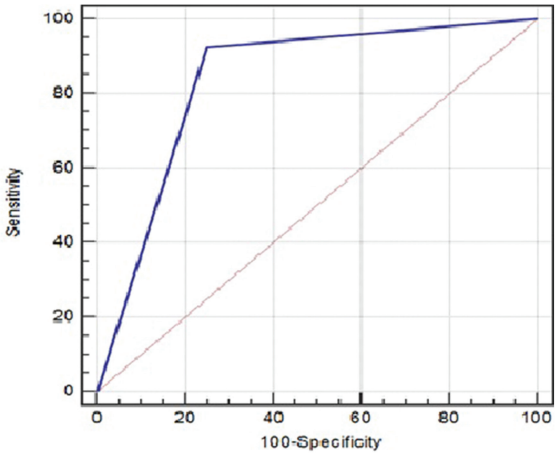
AFP, α-fetoprotein; AUC, area under the curve; DKK-1, dickkopf-1; HCC, hepatocellular carcinoma; LC, liver cirrhosis.

Figure 1



Receiver operating characteristic curves for dickkopf-1 and α-fetoprotein (AFP) in the diagnosis of hepatocellular carcinoma versus liver cirrhosis and control groups.

Figure 2



Receiver operating characteristic curves for the combination of dickkopf-1 and α-fetoprotein in the diagnosis of hepatocellular carcinoma versus liver cirrhosis and control groups.

differentiating HCC patients from high-risk patients. We found that DKK-1, at a cutoff value of more than 331 pg/ml had the best diagnostic performance with greater AUC (0.831), sensitivity (80.0%), specificity (87.5%), NPV (72.4%), and PPV (91.4%) than AFP, at a cutoff value of more than 8 IU/ml, with an AUC of 0.821, sensitivity of 77.5%, specificity of 83.3%, NPV of 69.0%, and PPV of 88.6%. There was no significant difference between the two markers in AUC (P = 0.981). The combination of the two markers got the best sensitivity (92.5%). There was no significant difference between the combination of DKK-1 and AFP compared with the use of a single marker either DKK-1 or AFP in AUC (P = 0.921 and 0.977, respectively (Table 4, Figs. 3 and 4).

When these two markers were used to diagnose early-stage HCC in cirrhotic patients, DKK-1 still showed better performance with the largest AUC (0.870) compared with AFP (0.808), suggesting that DKK-1 was better in distinguishing HCC, especially patients at an early stage, from high-risk patients. There was no significant difference between the two markers in AUC (P = 0.696).

The combination of two markers had the best sensitivity (85.0%) in differentiating patients with early HCC from high-risk cirrhotic patients. No significant difference could be found between the combination of DKK-1 and AFP compared with the use of a single marker either DKK-1 or AFP in AUC (P = 0.520 and 0.754, respectively).

Discussion

HCC is the second cause for cancer death in both sexes in Egypt [2].

The poor prognosis of HCC is due to the absence of symptoms in the early stages. More than 60% of HCC patients are diagnosed at a late stage when metastasis has developed [11], resulting in an overall 5-year survival rate of less than 16% [12].

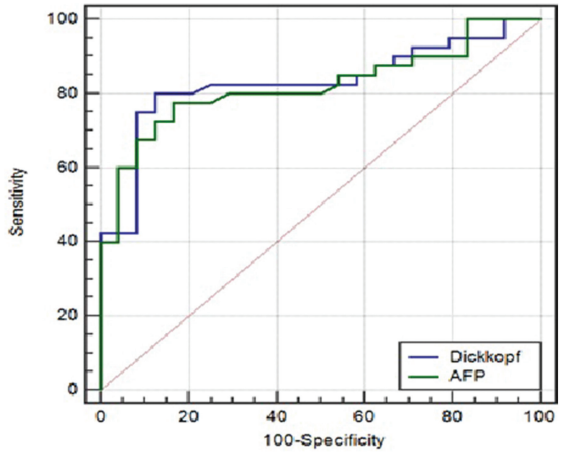
Shen *et al.* [13] suggest that DKK-1 may be a potential marker in the diagnosis of many types of cancers, and an elevated expression of DKK-1 is associated with the development of HCC.

Table 4 Performance of α-fetoprotein, dickkopf-1, and their combination for distinguishing hepatocellular carcinoma from high-risk patients (the liver cirrhosis group)

	Cutoff	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	AUC
HCC versus LC							
DKK-1	>331	80.0	87.5	91.4	72.4	82.81	0.831
AFP	>8	77.5	83.3	88.6	69.0	79.7	0.821
DKK-1 and AFP	AFP >8 or DKK-1 >331	92.5	70.8	84.1	85.0	84.4	0.817
Early-stage versus LC							
DKK-1	>331	70.0	87.5	82.4	77.8	79.6	0.870
AFP	>8	75.0	83.3	78.9	80.0	79.6	0.808
DKK-1 and AFP	AFP >8 or DKK-1 >331	85.0	70.8	70.8	85.0	77.3	0.779

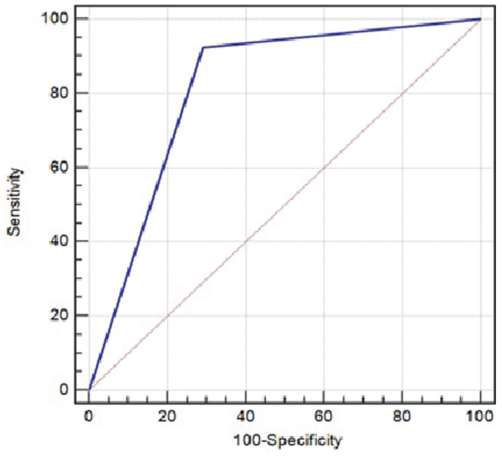
AFP, α-fetoprotein; AUC, area under the curve; DKK-1, dickkopf-1; HCC, hepatocellular carcinoma; LC, liver cirrhosis; NPV, negative predictive value; PPV, positive predictive value.

Figure 3



Receiver operating characteristic curves for dickkopf-1 and α-fetoprotein (AFP) in the diagnosis of hepatocellular carcinoma versus liver cirrhosis.

Figure 4



Receiver operating characteristic curves for the combination of dickkopf-1 and α-fetoprotein in the diagnosis of hepatocellular carcinoma versus liver cirrhosis.

This study was performed on 80 individuals and were divided into three groups: HCC group: 40 patients, LC group: 24 patients, and control group: 16 healthy persons.

In this study, DKK-1 levels were significantly elevated in the HCC group than the LC group (P < 0.001). Also, DKK-1 levels were significantly elevated in and could distinguish HCC patients from controls (P < 0.001). These results were in agreement with those of Ge *et al.* [14], Erdal *et al.* [15], Sharaf *et al.* [16], Fouad *et al.* [17], and Qi *et al.* [10].

Although DKK-1 inhibits Wnt/β-catenin signaling, its overexpression has no effect on HCC cells with cytoplasmic/nuclear β-catenin accumulation. This may be suggestive of an abrogated negative feedback loop, most likely due to genetic changes disrupting the multiprotein complex that controls' β-catenin stability [18]. It may also imply that the inhibition effect of DKK-1 is only functional until reaching a point of saturation beyond which it cannot exert its inhibitory effect [19].

In our study, we showed that AFP levels were significantly elevated in the HCC group than both cirrhotic and control groups (P < 0.001 and 0.001, respectively. This was in agreement with the results of Ge *et al.* [14] and Erdal *et al.* [15].

In this study, comparison between serum levels of DKK-1 and AFP in early-stage HCC (0 and A) and intermediate- and late-stage HCC (B, C and D) showed significant increased level of DKK-1 in stages B, C, and D than stages 0 and A (P = 0.006). However, AFP showed no significant difference (P = 0.350). The same results were reported by Kim *et al.* [18], Sharaf *et al.* [16], and Bakr *et al.* [20].

DKK-1 leads to an increase in the density of vessel wall and its diameter in adult rats. This might indicate DKK-1 effect in microvascular remodeling and tumor angiogenesis, and possibly accounting for DKK-1-mediated cancer growth promotion *in vivo* [18].

We evaluated the diagnostic accuracy of DKK-1 as a serological biomarker for HCC and found that

the performance of DKK-1 was better than AFP in distinguishing HCC patients from the nonmalignant group (LC and control groups) when the cutoff value was more than 331 pg/ml. DKK-1 showed a sensitivity and specificity of 80 and 87.5%, respectively (AUC 0.847). The same results were obtained by Ge *et al.* [14].

Although AFP shows a sensitivity and specificity of 77 and 85%, respectively, AUC was 0.830 when the cutoff value was more than 8 ng/ml. DKK-1 showed higher sensitivity, specificity, and AUC than AFP; however, it was not statistically significant. When using the currently recommended clinical cutoff for AFP (>20 ng/ml), the sensitivity was 50.0% and the specificity was 97.0% (AUC 0.738). In fact, the results of using two markers together were better than those of each marker alone. A combination of these two markers in the diagnosis of HCC versus the nonmalignant group (LC and control groups) improved the sensitivity (92.5%), whereas the specificity was decreased (75%) (AUC 0.838). The same results were obtained by Ge *et al.* [14].

As early detection is essential in improving the survival of HCC patients, we determined the diagnostic accuracy of these two markers in the early-stage HCC (BCLC stages 0 and A) versus the nonmalignant group (LC and control groups).The optimum cutoff for DKK-1 was more than 331 pg/ml with an AUC of 0.787, sensitivity of 70.0%, and specificity of 87.5%. However, the optimum cutoff for AFP was more than 8 IU/ml (AUC 0.821, sensitivity 75.0%, specificity 85.0%). Also, combining the two tumor markers improved the sensitivity (85%) but the specificity was decreased 75% (AUC 0.800). These results were nearly in line with those recorded by Ge *et al.* [14].

Most HCC cases resulted from LC and surveillance of cirrhotic patients is vital in the reduction of disease-related mortality [21]. So, we evaluated the diagnostic accuracy of both markers for differentiating early HCC from LC patients. When the cutoff value was more than 331 pg/ml DKK-1 showed a sensitivity and specificity of 80 and 87.5%, respectively (AUC 0.831). Similar results were obtained by Ge *et al.* [14]. Jang *et al.* [22] showed that a specificity of 80.5% an d a sensitivity of 50% of DKK-1 in diagnosing HCC versus LC at a cutoff of more than 500 pg/ml. Sharaf *et al.* [16] showed that the specificity was 96.6% but the sensitivity was 66.7% of DKK-1.

Although AFP showed a sensitivity and specificity of 77.5 and 83.3%, respectively, AUC was 0.821 when the cutoff value was more than 8 IU/ml. DKK-1 showed higher sensitivity, specificity, and AUC than AFP; however, it was not statistically significant. Similar results were obtained by Tianxiang *et al.* [14]. In contrast, Jang *et al.* [22] showed that sensitivity and specificity of AFP were more than DKK-1 at a cutoff more than 20 ng/ml. The combination of DKK-1 and AFP improved sensitivity (92.5%), whereas the specificity was decreased (70.8%). Similar results were obtained by Jang *et al.* [22].

Also, we evaluated the diagnostic accuracy of both markers in early HCC (BCLC stages 0 and A) versus LC. At an optimum cutoff for DKK-1 (>331 pg/ml), it showed a sensitivity of 70.0%, specificity of 87.5%, and an AUC of 0.870. At an optimum cutoff for AFP of more than 8 IU/ml, it showed an AUC of 0.88 and sensitivity of 75.0%, which was more than DKK-1 and a specificity of 83.3%, which was less than DKK-1. These results were in agreement with those of Ge *et al.* [14].

The combination of DKK-1 and AFP improved sensitivity (85.0%), whereas the specificity was decreased (70.8%) with an AUC of 0.779. These results were in agreement with those of Yang *et al.* [6] and Jang *et al.* [22].

Finally, it was found that serum DKK-1 level in peripheral blood samples obtained from HCC patients was a good marker for the detection of HCC. We also found that a significant increase in DKK-1 level in late stages (B, C, and D) more than early stages (0 and A), so this marker can be used to detect metastasis in patients with HCC. A combination of AFP and DKK-1 may be used as a panel for early diagnosis of HCC patients especially in cirrhosis.

Conclusion

DKK-1 levels were significantly higher in newly diagnosed HCC patients than in nonmalignant control group. The combination of the two markers (DKK-1 and AFP) enhanced the sensitivity.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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