

## Serum Dickkopf-1 as A Biomarker for The Diagnosis and Prognosis of Hepatocellular Carcinoma

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### ABSTRACT

**Background:** Hepatocellular carcinoma is one of the commonest cancers in world. It is one of the major health problems and its incidence is increasing. The main routinely used parameter for diagnosis of HCC is AFP, also it can be elevated in the liver cirrhosis. It represents a liver cell specific, not a tumor specific marker, for these reasons, we suggest use AFP as a supplementary marker for HCC diagnosis. So, identification of a sensitive biomarker to improve early diagnosis of HCC is in need.

**Aim of the work:** this study was aimed to evaluate the clinical significance of serum Dickkopf-1 (DKK1) as a diagnostic and prognostic marker in hepatocellular carcinoma. **Patients and Methods:** in this study 100 subjects were included and divided into 3 groups, Group I: included 50 patients with HCC, and divided into 2 subgroups according to Barcelona Clinic Liver Cancer (BCLC) into early HCC (24 patients) and late HCC (18 patients). Group II: included 25 patients with liver cirrhosis. Group III: included 25 healthy subjects (control). Clinical assessment, routine laboratory evaluation, CT scan and measurements of serum alpha-fetoprotein (AFP) and DKK1 were performed to all patients and repeated to group I patients 1 month after treatment.

**Results:** the current study showed that Serum DKK1 was significantly elevated in HCC group compared to liver cirrhosis and healthy control groups, with increased level in late than early stage. The optimum cut off values of DKK1 for diagnosis of HCC was 1.92 ng/mL (AUC 0.926, sensitivity 88% and specificity 83%). While the optimum cut off value for AFP was 102 ng/mL (AUC 0.904, 71% sensitivity and 84% specificity). Testing of both serum DKK1 and AFP increased the diagnostic accuracy for HCC (AUC 0.964, sensitivity 91%, and specificity 90%). Serum DKK1 level significantly decreases after HCC treatment with radio-frequency ablation.

**Conclusion:** It could be concluded that Testing of both serum DKK1 and AFP significantly increased the diagnostic accuracy for HCC. Meanwhile, DKK1 can be used alone for diagnosis of HCC even in HCC with inconclusive AFP. Serum DKK1 might be a potential diagnostic and prognostic marker for HCC.

**Keywords:** HCC patients, DKK1, AFP.

### INTRODUCTION

Hepatocellular carcinoma is the sixth most common malignant disease, and the third leading cause of cancer related death worldwide. HCC is prevalent in Asia and Africa, but it raises recently in the Western world due to an increase in hepatitis C virus infection <sup>[1]</sup>. In Egypt, Cancer liver forms 11.75% of the malignancies of all GIT organs and 1.6% of the total malignancies <sup>[2]</sup>.

Risk factors for HCC includes chronic hepatitis B virus and chronic hepatitis C infections, cirrhosis, chronic alcohol abuse, aflatoxin ingestion, non-alcoholic steatohepatitis (NASH) and metabolic liver diseases <sup>[3]</sup>.

Both HCV and HBV infections are most common risk factors for HCC among the Egyptian patients. 10%-20% of the general Egyptian population are HCV infected <sup>[4]</sup>.

80% - 90% of patients with HCC have underlying cirrhosis and the remaining 10% - 20% of the cases develop HCC without cirrhosis <sup>[5]</sup>.

HCC is disease with fast infiltrating growth and very poor prognosis <sup>[6]</sup>. The commonly used

screening method for liver cancer are ultrasound examination (US) of the liver and determination of

serum AFP levels <sup>[7]</sup>. Abdominal ultrasound is better, simple and easy method for the detection of HCC but it is an operator dependent and many focal lesions could be missed <sup>[8]</sup>.

AFP has approximately 60% specificity and 40% sensitivity for diagnosis of HCC, since minor elevations are common in the patients with chronic liver diseases, cirrhosis, germ cell tumor and in pregnancies <sup>[9]</sup>.

So, it is necessary to find specific and sensitive marker for early diagnosis of HCC, and for monitoring of the treatment response.

Dickkopf-1 (DKK1) is secretory protein which was first identified in 1998. DKK1 is an inhibitor of Wnt/ $\beta$ -catenin signaling pathway and a downstream target for  $\beta$ -catenin <sup>[10]</sup>.

The Wnt/ $\beta$ -catenin signaling pathway plays the main role in the development of both normal liver and hepato-carcinogenesis <sup>[11]</sup>.

It is hardly expressed in the normal human adult tissue except in placenta and embryonic tissue <sup>[12]</sup>.

DKK1 is up-regulated in various cancers including lung, breast, ovarian, prostatic cancers and HCC [13].

**AIM OF THE WORK**

This study was aimed to evaluate the clinical significance of serum Dickkopf-1 (DKK1) as a diagnostic and prognostic marker in hepatocellular carcinoma.

**PATIENTS and METHODS**

This cross-sectional study included a total of 75 patients with HCC and liver cirrhosis and 25 age-matched normal control individuals attending at Al-Azhar University Hospitals. Approval of the ethical committee and a written informed consent from all the subjects were obtained. This study was conducted between May 2016 and September 2018.

The 100 subjects included in this study were divided into 3 groups:

**Group I:** included 50 patients with HCC, and divided into 2 subgroups according to BCLC into **early HCC** (24 patients) and **late HCC** (18 patients).

**Group II:** included 25 patients with liver cirrhosis.

**Group III:** included 25 healthy subjects (control). Clinical assessment, routine laboratory evaluation, CT studies and measurement of serum alpha-fetoprotein (AFP) and DKK1 were performed to all patients and repeated to group I patients 1 month after treatment.

**RESULTS**

Serum DKK1 and AFP were significantly elevated in group I compared to group II and III (P<0.01) (Table 1), Mean serum DKK1 level in group I, II and III was 2.41 ± 1.12, 0.36 ± 0.05 and 0.31 ± 0.02 ng/ml respectively, and Mean serum AFP level in group I, II and III was 657 ± 433, 36 ± 18 and 6 ± 3 ng/ml respectively (Figure 1,2).

Comparison between Group II and III as regard serum DKK1 and AFP levels showed that there is significant difference as regard serum AFP (P<0.01), but no significant difference as regard to serum DKK1 (P>0.1) (Table 2). Comparison between (Early HCC; BCLC 0,A) and (Late HCC; BCLC B,C) as regard serum DKK1 and AFP levels showed that there is statistically significant difference as regard serum DKK1 and AFP (P<0.01) (Table 3).

Comparison between Very Early HCC (BCLC 0) and Group II as regard serum DKK1 and AFP levels showed that there is statistically significant difference as regard serum DKK1 (P<0.01) (Table 4), But no significant difference as regard serum AFP (P>0.1).

Comparison between before and after treatment with RF (curative) as regard serum DKK1 and AFP levels showed that there is statistically significant difference as regard serum DKK1 (P<0.05), But no significant difference as regard serum AFP (P>0.1). Comparison between before and after treatment with TACE (palliative) as regard serum DKK1 and AFP levels showed that There is no statistically significant difference as regard serum DKK1 and AFP (P>0.1).

The diagnostic performance of serum AFP and serum DKK1 when individually used for the discriminating of Group I from Group II and Group III, showed that the optimum diagnostic cut off value for DKK1 was 1,92 ng/mL with 88% sensitivity and 83% specificity while, the cut off value for AFP was 102 ng/mL with 71 % sensitivity and 84% specificity.

Combined use of both serum DKK1 and AFP increased the diagnostic accuracy for HCC compared with either of test alone (AUC 0.964, sensitivity 91%, and specificity 90%) (Table 5,6 and Figure 3).

**Table (1):** Statistical comparison between study groups as regard serum DKK1 and AFP levels

	<b>Group I (HCC) [n=50]</b>	<b>Group II (Liver Cirrhosis) [n=25]</b>	<b>P value</b>	<b>Group I (HCC) [n=50]</b>	<b>Group III (Control) [n=25]</b>	<b>P value</b>
<b>DKK1(ng/ml)</b>	2.41 ± 0.12	0.36 ± 0.05	<b>&lt;0.01*</b>	2.41 ± 0.12	0.31 ± 0.02	<b>&lt;0.01*</b>
<b>AFP (ng/ml)</b>	657 ± 133	36 ± 8	<b>&lt;0.01*</b>	657 ± 33	6 ± 1	<b>&lt;0.01*</b>

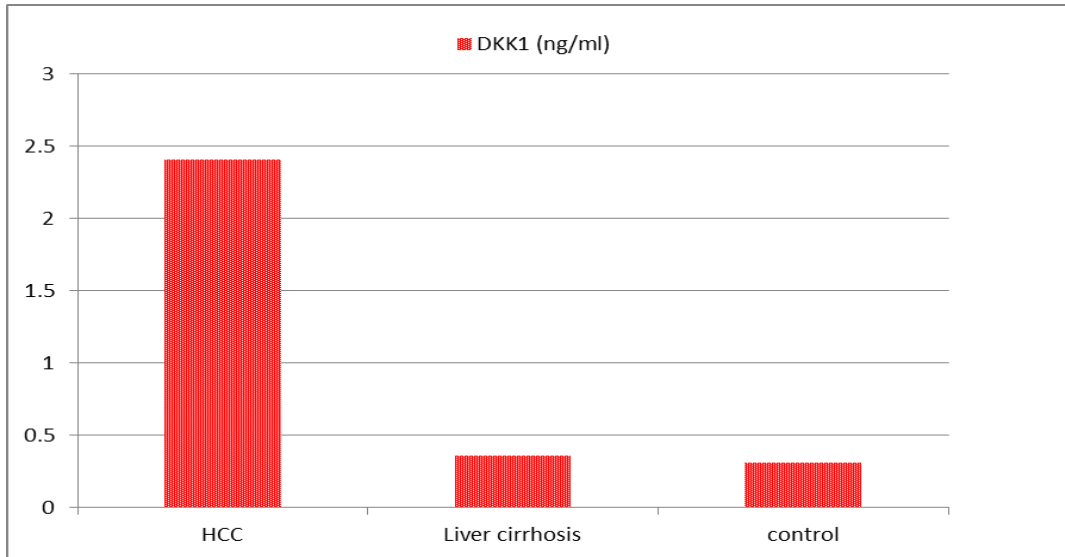
**Table (2):** Statistical comparison between Group II (liver cirrhosis) and Group III (control) as regard serum DKK1 and AFP levels

	<b>Group II (Liver Cirrhosis) [n=25]</b>	<b>Group III (Control) [n=25]</b>	<b>P value</b>
<b>DKK1 (ng/ml)</b>	0.36 ± 0.05	0.31 ± 0.02	>0.1
<b>AFP (ng/ml)</b>	36 ± 8	6 ± 1	<b>&lt;0.01*</b>

**Table (3):** Statistical comparison between subgroup IA (Early HCC; BCLC 0,A) and subgroup IB (Late HCC; BCLC B,C) as regard serum DKK1 and AFP levels

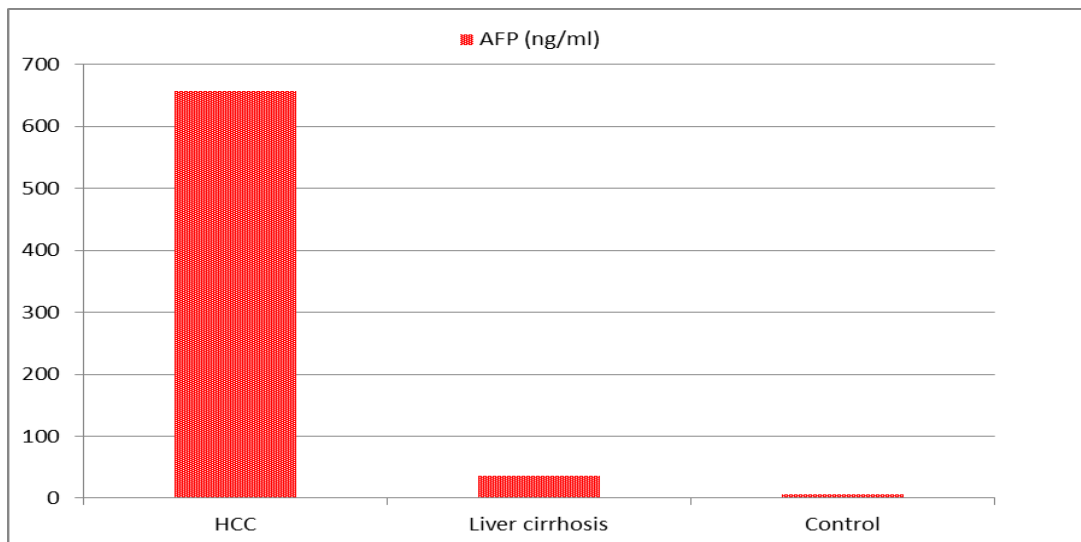
	Subgroup IA Early HCC [n=24]	Subgroup IB Late HCC [n=18]	P value
<b>DKK1 (ng/ml)</b>	1.72 ± 0.28	3.61 ± 0.34	<0.01*
<b>AFP (ng/ml)</b>	76 ± 3	866 ± 33	<0.01*

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**Figure (1):** Mean serum DKK1 levels in HCC, liver cirrhosis and healthy control groups

**Figure (2):** Mean serum AFP levels in HCC, liver cirrhosis and healthy control groups



**Table (4):** Statistical comparison between Very Early HCC (BCLC 0; single HFL<2cm) and Group II (liver cirrhosis) as regard serum DKK1 and AFP levels

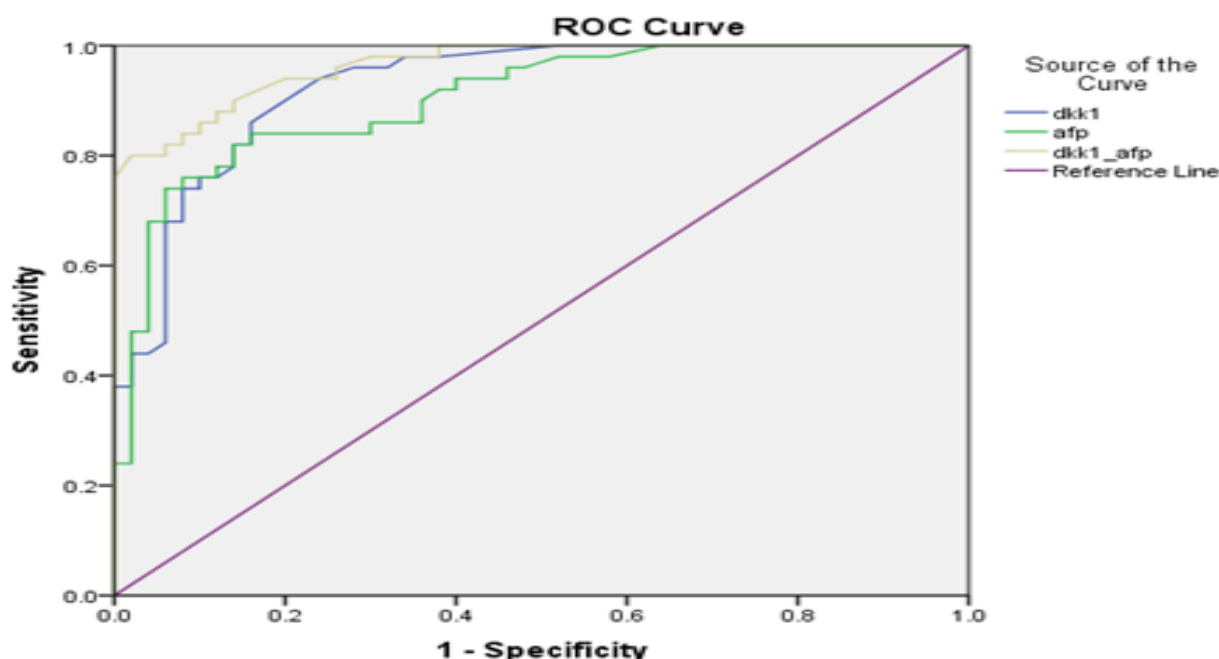
	Very Early HCC (BCLC 0) [n=6]	Group II (Liver Cirrhosis) [n=25]	P value
<b>DKK1 (ng/ml)</b>	1.46 ± 0.12	0.36 ± 0.05	<0.01*
<b>AFP (ng/ml)</b>	42 ± 4	36 ± 8	>0.1

**Table (5):** Diagnostic performance of serum DKK1 and serum AFP when individually used for discriminating Group I (HCC) from Group II (Liver Cirrhosis) and Group III (Control).

	AUC (95%CI)	ROC Cutoff	Diagnostic Sensitivity (%)	Diagnostic Specificity (%)	PPV (%)	NPV (%)
AFP (ng/mL)	0.904	102	71%	84%	82%	66%
DKK1 (ng/mL)	0.926	1,92	88%	83%	86%	78%

**Table (6):** Diagnostic performance of combined use of both serum DKK1 and serum AFP for Discriminating Group I (HCC) from Group II (Liver Cirrhosis) and Group III (Control).

	AUC (95%CI)	Diagnostic Sensitivity (%)	Diagnostic Specificity (%)	PPV (%)	NPV (%)
Combined AFP+DKK1	0.964	91%	90%	92%	88%



**Figure (3):** Multi-ROC curve analysis showing the diagnostic performance of the combined use of DKK1 (ng/mL) and AFP (ng/mL) in discriminating Group I (HCC) from Group II (Liver Cirrhosis) and Group III (Control), compared to their individual ROC curves.

## DISCUSSION

Hepatocellular carcinoma is one of the most common primary malignancies of the liver, forming about 85-90% of the all primary liver cancers [14]. 80%-90% of patients with liver cirrhosis will progress to HCC, HCV infection was present in 27% - 70% and HBV infection in 12%-50% of cases [15]. Early detection of HCC gives the opportunity for curative treatment which can prolong survival, Despite the vigorous trials to screen for early HCC by using serum AFP and abdominal ultrasound, early HCC is asymptomatic and the most cases are presented late at which the surgical treatment are not valuable [16]. 30-47% of patients with HCC are AFP negative and abdominal ultrasound used in the detection for HCC is suboptimal [17]. As a result, the widespread use of

serum AFP and ultrasound for surveillance of HCC has been questioned.

The Wnt/ $\beta$ -catenin signaling pathway plays an important role in development of both normal liver

and hepato-carcinogenesis [18]. The DKK family are a secreted glycoproteins and have ability in regulating Wnt/ $\beta$ -catenin. DKK-1 is the most studied member of DKK family, it is rarely expressed in normal tissue except in placental and embryonic tissues [19]. *Shen et al.* [20] suggest that, DKK1 may be potential marker in the diagnosis of many types of cancers, and elevated expression of DKK1 is associated with development of HCC. Also, they reported the diagnostic accuracy of DKK1 as serum marker for HCC diagnosis in large-scale, multicenter study. The study documented that, serum DKK1 has a high sensitivity and

specificity in the diagnosing of HCC, especially early stages and HCC cases with normal  $\alpha$ -fetoprotein (AFP). They also demonstrated that the combination of both serum  $\alpha$ -fetoprotein with DKK1 could further improve the diagnostic accuracy.

Our study revealed higher incidence of HCC in males than in females (80% and 20% respectively), male:female ratio was 4:1, in which was also published by **El-Serag and Rudolph** [14]. Serum DKK1 it was significantly higher in group I compared with group II and group III ( $p < 0.01$ ). But there was no significant difference in group II compared with group III ( $p > 0.1$ ). These results are in agreement with **Shen *et al.*** [20], who found that levels of DKK1 in serum were significantly higher in patients with HCC than in liver cirrhosis and healthy control groups. They found also that DKK1 were not significantly higher in liver cirrhosis patients in comparing to healthy control group.

In our study, serum DKK1 and AFP were significantly higher in subgroup IB (late HCC) compared with subgroup IA (early HCC) ( $p < 0.01$ ), so DKK1 has a prognostic value. Our results are in agreement with **Hoda *et al.*** [21] who assessed serum DKK1 according to BCLC staging of HCC, they found a significantly increased serum DKK1 level in patients with BCLC (C,D) than BCLC (A,B) stage. Another study by **Yu *et al.*** [22] found that Dkk1 was significantly elevated in multi-nodular HCC (multiple lesions) with high metastatic potential comparing to solitary HCC. In the present study, serum DKK1 was significantly higher in very early HCC (single HFL < 2cm) group comparing with group II ( $p < 0.01$ ). But there was no significant differences in both groups as regard serum AFP ( $p > 0.1$ ). These results agreed with **Hoda *et al.*** [21] who found significant elevations of serum DKK1 levels in cases with HCC less than 2 cm in diameter (single HFL) when comparing to healthy control, and the levels was increased significantly to be higher among lesions > 5cm comparing to smaller one (< 2cm). As regard serum DKK1 and AFP before and after curative treatment with RF, there were significant differences between both groups as regard DKK1 ( $p < 0.05$ ), But no significant difference between both groups as regard AFP ( $p > 0.1$ ). On the other hand, serum DKK1 and AFP before and after palliative treatment (TACE), there were no significant difference in both groups ( $p > 0.1$ ). Our results are in agreements with **Sharaf *et al.*** [23] who found that there were significant differences between the patients treated with either radiofrequency ablation (RF) or ethanol injection (EI) as regards AFP and DKK1, there was decrease in the mean level of both serum markers after treatment ( $p < 0.001$ ).

Assessment of diagnostic performance of both serum DKK1 and AFP when used in

discriminating Group I (HCC) from Group II (Liver Cirrhosis) and Group III (Control), revealed a better diagnostic performance of DKK1 (AUC 0.926) compared to AFP (AUC 0.904). The best ROC cut-off for DKK1 was 1,92 ng/mL, this had a diagnostic sensitivity of 88%, specificity 83%, positive predictive value 86%, negative predictive value 78%. The best ROC cut-off for AFP was 102 ng/mL, this had a diagnostic sensitivity of 71%, specificity 84%, positive predictive value 82%, negative predictive value 66%. Our results are in agreement with **Ge *et al.*** [24] who reported the better diagnostic performance in the case of DKK1 (AUC 0.889) compared to AFP (AUC 0.831).

Their best cut-off for serum DKK1 was 1,31 ng/mL, this had diagnostic sensitivity of 79.8% and specificity 89.4%, and the best ROC cut-off for AFP was 6.8 ng/mL, this had a diagnostic specificity 88.0% and sensitivity of 71.9%. Assessment of diagnostic performance of combined use of both serum DKK1 and AFP in discriminating Group I (HCC) from Group II (Liver Cirrhosis) and Group III (Control), revealed a better diagnostic performance than that of DKK1 or AFP alone with (AUC 0.964), sensitivity was 91%, specificity 90%, positive predictive value 92%, negative predictive value 88%. Our results are agree with **Ge *et al.*** [24] who reported a better diagnostic performance of the combined use of both markers as evidenced by the increased in the AUC to reach 0.931 vs 0.831 for AFP and 0.889 for DKK1 when individually used, this had a diagnostic specificity of 87.7%, and sensitivity of 88.8%. Also our results are in accordance with **Shen *et al.*** [20] who reported that the combination of both AFP and DKK1 could further improve the diagnostic accuracy of hepatocellular carcinoma.

Concerning our evaluation of the diagnostic utility of serum DKK1 and serum AFP in discriminating early HCC (BCLC 0, A) from late HCC (BCLC B, C), our results revealed a good discriminating ability for DKK1 where the (AUC was 0.964). The best cut-off was 2,85 ng/mL with diagnostic sensitivity of 93%, specificity 90%, positive predictive value 91%, negative predictive value 85%. Concerning serum AFP, this showed a lower performance (AUC was 0.942). The best cut-off was 477 ng/mL with a diagnostic sensitivity of 82%, specificity 91%, positive predictive value 87%, negative predictive value 83%. These results agreed with **Kim *et al.*** [25] who found that serum DKK1 is efficient in the discrimination of early from late HCC.

## CONCLUSION

This study showed that serum DKK1 may serve as good diagnostic biomarker for the HCC diagnosis particularly at early stages, also DKK1 alone or in combination with AFP can significantly

improve the diagnostic accuracy of early HCC with sensitivity, specificity and accuracy higher than of AFP alone. DKK1 may serve as good prognostic biomarkers for HCC which evidenced by statistically significant differences between its level in early and late stages of HCC. DKK1 could be a promising marker for prediction of complete and partial response to treatment either curative (RF) or palliative (TACE).

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