

Original Research Article

Diagnostic Study of Serum Dickkopf 1 Level and Alpha Fetoprotein L3 as

Tumor Biomarkers in Hepatocellular Carcinoma

Comment [Ma1]: Needed to be changed

Abstract

Background: Alpha Fetoprotein (AFP) is now the most frequently applied tumour biomarker for the early diagnosis and clinical follow-up of HCC patients. Dickkopf 1 (DKK 1) is a protein that has a role in embryonic head morphogenesis. Several investigations revealed that DKK 1 regulates a variety of pathological and physiological processes. The aim of this work was to evaluate the diagnostic role of serum DKK 1 level and AFP- L3 as tumor markers in hepatocellular carcinoma.

Methods: This observational research enrolled 60 participants aged above 18 years, group 1 include 10 healthy control participants, group 2 involved 25 cirrhotic cases and group 3 involved 25 cases diagnosed with HCC. All cases underwent taking of full history, clinical assessment, radiology, laboratory examination and specific investigations such as the tested two markers AFP-L3 and DKK1.

Results: AFP-L3 had 84% sensitivity and 67.7% specificity, while DKK1 had sensitivity and specificity of 76% and 80% respectively to differentiate between cirrhotic and HCC cases, using a cut-off value of 8.62 ng/ml. two markers combination had specificity and sensitivity of 92%, 72% respectively and increase the accuracy for detection of HCC up to 82%.

Conclusions: Both AFP-L3 and DKK1 could act as surrogate biomarkers for HCC and combination of two markers should be kept in mind to reach the optimum accuracy.

Keywords: Dickkopf 1, Alpha Fetoprotein L3, Tumor Biomarkers, Hepatocellular Carcinoma.

Comment [Ma2]: Age range should be written (including number of males and females) for both patients and control.

Introduction:

Hepatocellular carcinoma (HCC) is a frequent solid tumour and the third main cause of Mortality due to cancer globally. Chronic HBV infection cases, particularly those having chronic liver disease and cirrhosis, have a greater risk of having HCC ^[1].

Most instances of HCC are identified in later phases, leading to a dismal prognosis with a survival rate over the course of five years of fewer than 5% ^[2].

Alpha Fetoprotein (AFP) is now the most widely applied tumour biomarker for the earlier diagnosis and clinical follow-up of HCC cases with 41-65% sensitivity and 80-94% specificity even 20 ng/ml cut-off value ^[3].

Globally, an AFP at 200 ng/mL cutoff value reveals the presence of HCC. Additionally, acute and chronic viral hepatitis, as well as cases of hepatitis C-related cirrhosis, may be accompanied by modestly elevated AFP levels ^[4].

Due to its low positive rate, false-positive findings, and eventually false-negative results, this extensively used marker does not provide satisfying results in the early HCC detection, hence restricting its general use ^[5].

Lens culinaris agglutinin-reactive AFP, or AFP-L3, is a highly specific isoform of AFP for HCC. AFP-L3 is provided as a percentage of AFP-L3 relative to the complete level of AFP ^[6].

The protein Dickkopf1 (DKK1) is important in head morphogenesis during embryonic development. Several investigations revealed that DKK1 regulates a variety of pathological and physiological functions, such as adult hippocampal neurogenesis ^[7], osteoclastogenesis^[8], proliferation of tumor cells, migration, invasion, and survival ^[9].

DKK1 expression is diminished in human colon cancers, while overexpression was found in multiple myelomas, Wilms' tumors, and human hepatoblastomas ^[10]. DKK1 was shown to be

selectively overexpressed as a secreted protein in cancer cells, and it has the potential to be utilized as biomarker of serum for many human malignancies ^[11].

This work aimed to evaluate the diagnostic role of serum DKK 1 level and AFP- L3 as tumor markers in HCC.

Patients and Methods:

This observational research enrolled 60 participants aged above 18 years, group 1 include 10 healthy control participants, group 2 involved 25 cirrhotic cases and group 3 involved 25 cases diagnosed with HCC. This study was performed at clinical pathology and internal medicine departments- Tanta university hospital. The study was done after approval from Local ethics committee, Faculty of Medicine, Tanta University. Before including, any participant in the study, signed informed consent was acquired from all of them.

Exclusion criteria were other malignant diseases, prior treatment of HCC either surgical, interventional or medical, autoimmune diseases, prior liver transplant, benign biliary disease e.g. (first-degree biliary cholangitis, primary sclerosing cholangitis), malignant biliary disease (e.g., cholangiocarcinoma) and pregnancy.

All cases underwent: full history taking, clinical evaluation, radiology like abdominal US for the evaluation of liver, portal vein and/or ascites, spleen, and triphasic CT abdomen for diagnosis of HCC. Laboratory analysis such as complete blood count, liver function tests as serum albumin, total serum bilirubin, liver enzymes (SGPT, SGOT), prothrombin time and INR, kidney function test such as serum creatinine, AFP by ELISA and specific investigations including the tested two markers by ELISA: AFP-L3 and DKK1.

Principle of AFP-L3 test ^[12]:

This assay utilizes the sandwich quantitative enzyme-linked immunosorbent assay (ELISA) to measure the concentration of AFP-L3 in samples. On a microplate, AFP L3-specific

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antibodies (Ab) were precoated. We pipetted standards and samples with a Horseradish Peroxidase (HRP)-conjugated antibody specific for AFP L3 into the wells.

Principle of DKK1 test:

Using a double-antibody sandwich ELISA, the kit determined the concentration of DKK1 in samples. DKK1 is introduced to a monoclonal antibody Enzyme well that has been precoated with DKK1 monoclonal antibody, followed by the addition of biotin-labeled DKK1 antibodies, coupled with Streptavidin-HRP to produce the immunological complex; Afterwards, washing and incubation are repeated to eliminate the uncombined enzyme. The correlation between the chroma of colour and the level of Human Substance Dickkopf 1 (DKK1) in a sample is positive.

Statistical analysis

Statistical analysis was performed by SPSS v26 (IBM Inc., Chicago, IL, USA). Quantitative variables were expressed as mean and standard deviation (SD), non-parametric data was presented as median (min – max), Kolmogorov-Smirnov test was used to test quantitative data for normality and compared between the two groups utilizing ANOVA (F) test. Spearman's correlation was applied to examine the correlation between two variables given nonparametric quantitative data. Qualitative variables were presented as frequency and percentage (%) and were analysed utilizing the Chi-square test. Kruskal Wallis test (KW) was applied to compare between groups. ROC curve was applied to assess the diagnostic accuracy of the markers. A two-tailed P value less than or equal to 0.05 was considered statistically significant.

Results:

Males were statistically significantly higher in HCC group than the other two groups ($p = 0.002$). There was a significant increase between the three groups regarding **diabetes** and **smoking** ($p = 0.032$ and 0.001 respectively). There was a statistically significant difference

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regarding, splenomegaly, ascites, jaundice, lower limb edema, history of Encephalopathy and Hematemesis and melena between the Cirrhosis and HCC groups in comparison to control group ($p < 0.05$). Regarding age and hypertension, there was insignificant difference among the investigated groups. Table 1

Comment [Ma6]: All information or parameters labelled in pink should be mentioned in the methodology (if data collected in questionnaire, or parameters measured in the lab).

Table 1: Comparison of the demographic data, comorbidities, risk factors and clinical data within the study groups

Variable	Control group N= 10	Cirrhosis group N= 25	HCC group N= 25	P value	
Age/years	50.40 ± 11.6 a	49.70 ± 6.8a	52.15 ± 7.9a	0.162	D2
Gender	Males	4 (40%)	11 (44%)	0.002*	D1
	Females	6 (60%)	14 (56%)		
Diabetes	2 (20%) a	13 (52%) b	15 (60%) b	0.032*	D2
Hypertension	3 (30%) a	9 (36%) a	13 (52%) a	0.124	D2
Smoking	1 (10%) a	6 (24%) b	14(56%) c	0.001*	D1
Splenomegaly	NA (a)	20 (80%) (b)	21 (84%) (b)	0.011*	D2
Ascites	NA(a)	12 (48%) (b)	17 (68%) (b)	0.001*	D2
Jaundice	NA (a)	11 (44%) (b)	10 (40%) (b)	0.001*	D2
Lower limb edema	NA (a)	10 (40%) (b)	15 (60%) (b)	0.001*	D2
History of Encephalopathy	NA (a)	10 (40%) (b)	15 (60%) (b)	0.001*	D2
History of Hematemesis/ Melena	NA (a)	10 (40%) (b)	15 (60%) (b)	0.005*	D2

Data are presented as mean ± SD or frequency (%), * statistically significant if $P < 0.05$, a, b, c: Similar letters indicate no statistically significant difference between the adjacent groups different letters indicate presence of statistically significant difference between the adjacent groups, D1: Indicates presence of statistically significant difference between HCC and cirrhotic groups, D2: Indicates presence of statistically insignificant difference between HCC and cirrhotic groups. HCC: Hepatocellular carcinoma.

All of the tested basic laboratory parameters showed a significant difference between the three groups ($p < 0.05$) except total leucocytic count. Serum albumin had a significant decrease in the cirrhotic and HCC groups. Conversely, total serum bilirubin showed a significant increase in cirrhotic and HCC groups. Both liver transaminases showed a significant elevation in the two diseased groups compared to controls. Creatinine showed no significant difference between the three groups. Table 2

Table 2: Comparison of the laboratory investigations within the study groups

Variable	Control group N= 10	Cirrhosis group N= 25	HCC group N= 25	P value	
HB gm/dl	(12.1-15.1) 13.5 a	(9.3-11.9) 10.7 b	(8.1-12.2) 9.6 c	0.001*	D1
PLTs (10 ³)	(163-324) 251 a	(29-100) 54 b	(30-126) 59 b	0.001*	D2
WBCs (10 ³)	(4.6-10.3) 6.05 a	(2.6-18.6) 6.26 a	(2.6-10.3) 6.2 a	0.331	D2
Creatinine mg/dl	(0.51-1.1) 0.86 a	(0.35-1.3) 0.92 a	(0.45-1.4) 0.85 a	0.360	D2
INR	(1- 1.2) 1.05 a	(1.2-2.3) 1.5 b	(1.1-2.4) 1.6 b	0.001*	D2
Albumin gm/dl	(3.9-5.1) 4.4 a	(1.9-3.7) 2.6 b	(2.1-3.8) 2.7 b	0.001*	D2
Total bilirubin mg/dl	(0.3-0.9) 0.65 a	(0.4-21.7) 4.4 b	(0.6-20.6) 3.8 b	0.001*	D2
Direct bilirubin mg/dl	(0.15-.9) 0.56 a	(0.3-17.6) 3.9 b	(0.4-17.6) 3.2 b	0.001*	D2
ALT U/l	(19-40) 33 a	(10-70) 43.5 b	(16-210) 46.5 b	0.010*	D2
AST U/l	(22-40) 34 a	(10-97) 57.5 b	(28-320) 57 b	0.001*	D2

Data are presented as median (IQR). * statistically significant if P <0.05 a, b, c: Similar letters indicate no statistically significant difference between the adjacent groups different letters indicate presence of statistically significant difference between the adjacent groups.; D1: Indicates presence of statistically significant difference between HCC and cirrhotic groups.; D2: Indicates presence of statistically insignificant difference between HCC and cirrhotic groups. HB: Haemoglobin, PLTs: Platelets, WBCs: White blood cells, INR: International normalized ratio, ALT: Alanine transaminase. AST: Aspartate aminotransferase.

Both AFP, AFP-L3 and AFP-L3 index (%) showed a significant elevation in the HCC group compared to both cirrhotic and control groups (p < 0.001). DKK1 a significant increase in HCC group compared to the other two groups (p < 0.001). Table 3

Table 3: Comparison of levels of AFP, AFP-L3, AFP-L3 index and DKK 1 within the study groups

AFP level ng/ml	(1.4-16.4) 4.7 a	(20.6-260.3) 192.4 b	(53.4-1468.5) 309.3 c	0.001*	D1
AFP-L3 level ng/ml	(0.15-0.61) 0.36 a	(1.96 – 32.38) 17.84 b	(8.54-68.45) 43.15 c	0.001*	D1
AFP-L3 index (%)	(2.2-9.3) 7.65 a	(0.9 – 12.3) 9.27 a	(6.25-21.45) 13.95 b	0.05*	D1
DKK 1 level (ng/ml)	7.28 ±1.10 a	8.13± 0.89 a	10.73 ± 3.19 b	0.001*	D1

Data are presented as mean ± SD and median (IQR). * statistically significant if P <0.05 a, b, c: Similar letters indicate no statistically significant difference between the adjacent groups different letters indicate presence of statistically significant difference between the adjacent groups.; D1: Indicates presence of statistically significant difference between HCC and cirrhotic groups.; D2: Indicates presence of statistically insignificant difference between HCC and cirrhotic groups. AFP: Alpha-fetoprotein, DKK 1: Dickkopf WNT signaling pathway inhibitor 1.

At 24.83 ng/ml cut-off value, AFP-L3 had 84% sensitivity and 67.7% specificity, while DKK1 had 76% sensitivity and 80% specificity to differentiate between cirrhotic and HCC cases, using a cut-off value of 8.62 ng/ml. Combination of two markers had specificity and sensitivity of 92%, 72% respectively and increase the accuracy for detection of HCC up to 82%. Figure 1

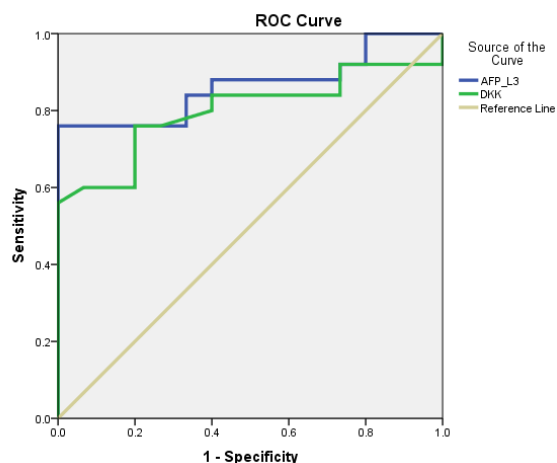


Figure 1: ROC curve to detect the cutoff value of AFP-L3 and DKK1 to differentiate between cirrhosis and HCC cases

Discussion

HCC is a prevalent solid tumour and the third greatest cause of worldwide cancer-related mortality ^[1]. HCC is the second main cause of Cancer-related mortality among both sexes in Egypt ^[13]. A novel serologic marker with adequate sensitivity and specificity is needed for early HCC detection ^[14-16].

In the present investigation, AFP levels were significantly higher in the HCC group than in the cirrhotic and control groups ($p < 0.001$). This came in line with Yang et al. ^[17] who reported that Serum AFP concentration in HCC cases were considerably greater than those in cases with chronic hepatitis, liver cirrhosis, and healthy controls.

A recent research by Dala and colleagues ^[18], also documented significantly higher AFP concentrations in HCC and cirrhotic cases vs. controls. Additionally, it was obvious that

HCC cases express significantly higher levels of this marker than cirrhotic cases ($p = 0.004$).

This was also in harmony with the findings of Ge et al.^[19] and Erdal et al.^[20].

In this research, AFP-L3 serum levels were significantly higher in the HCC group vs. the cirrhotic and control groups ($p < 0.001$). It has been observed that AFP-L3 is more sensitive than AFP for small or first stage HCC^[21]. The study conducted by *Khien et al., 2001* revealed that Although AFP-L3 is recognised for its excellent specificity for HCC, it also exhibits tumour characteristics like lack of differentiation or malignant metastasis^[22].

Fariad et al.^[23] reported that AFP-L3 had a significant elevation of in HCC cases than cirrhotic cases and controls ($p < 0.001$).

In the present research, there was significantly higher AFP L3 index in the HCC group compared to the cirrhotic and control groups ($p < 0.001$).

Choi and his colleagues^[24] and Park et al.^[25] stated that AFP L3 index is significantly higher in the HCC group compared to the cirrhotic and control groups ($p < 0.001$).

In the present study, at a cut-off value of 24.83 ng/ml, AFP-L3 had 84% sensitivity and 67.7% specificity for detecting cases with HCC. Multiple studies confirmed the previous findings. In a previous study, AFP-L3 had sensitivity and specificity of 80% for detecting HCC at a cutoff value of 29 ng/ml^[23].

In this research, DKK1 had a high significant difference between the three groups ($p < 0.001$). It was evident that HCC cases expressed significantly higher values of that marker.

Awad et al.^[26] and Fouad et al.^[27] confirmed the findings of this study. Serum DKK1 had significant elevation in HCC group vs. cirrhotic cases and controls ($p < 0.05$). On the contrary, the same study reported no significant difference between HCC and cirrhotic cases^[20].

The current study also showed no significant difference between DKK1 levels in cirrhotic cases against controls ($p > 0.05$). Shen et al. [28] and Erdal et al. [20] stated that serum DKK1 was statistically similar between cirrhotic and control groups.

In the current study, DKK1 had 76% sensitivity and 80% specificity to differentiate between cirrhotic and HCC cases, at a cut-off value of 8.62 ng/ml.

Likewise, Awad and his colleagues [26] reported that serum DKK1 had sensitivity and specificity of 87.3% and 82.9% respectively for detecting HCC cases, at a cutoff value of 8.92 ng/ml.

In 2019, another study used a cutoff value of 1.122 ng/ml for the HCC diagnosis of cases with 80% sensitivity and 77.1% specificity [18].

Hassan et al. [29] reported that DKK1 level of 331 pg/ml had sensitivity and specificity of 80 and 87.5% respectively for identifying cases with HCC. The diagnostic accuracy of that marker was 81.3% in that study.

A recent study conducted by S.H. Choi et al. [30] documented that DKK1 promoted angiogenesis via an-independent upregulation of vascular endothelial growth factor receptor 2. Furthermore, DKK1 increased tumour cell proliferation in animal models by enhancing the establishment of vasculogenic mimicry [31]. What is interesting in this study is that combination of serum DKK1 and AFP L3 increase the specificity of detection of HCC to 92%.

Comment [Ma7]: Explain.

It is evident that there is marked heterogeneity in the existing literature regarding both markers, levels, and optimum cutoff values, and that is reasonable. This difference could be explained by difference in sample size, testing methods, and statistical tests.

Limitations: first of all, this is single-center research with a rather modest sample size. Also, the level of both markers should be correlated with disease stage but the sample cases in the HCC was small and not sufficient to be divided according to Child Pugh classification into

three subgroups. Therefore, these disadvantages should be thoroughly addressed in further research.

Conclusions:

Both AFP-L3 and DKK1 could act as surrogate biomarkers for HCC and combination of two markers should be kept in mind to reach the optimum accuracy.

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