# The Potential Role of Dickkopf-1 and β-catenin as Biomarkers for the Diagnosis of Hepatocellular Carcinoma Patients

*Thesis*

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# Abstract

The aim of the present work is to investigate if serum β-catenin and Dickkopf-1 (DKK1) levels can predict the progression of chronic hepatitis C (CHC) into hepatocellular carcinoma (HCC) at early stages. The study was conducted on 187 individuals including 37 normal adults as reference controls, and a total of 150 adult patients with CHC were divided into 3 main groups according to liver involvement: HCV without cirrhosis (CHC), patients with liver cirrhosis (LC) and HCC patients. Liver functions, HBs Ag, HCV antibodies were assayed. Alpha-fetoprotein (AFP), AFP-L3, β-catenin and DKK-1 were assayed by ELISA technique. DKK1 levels were significantly increased ($p < 0.001$) in the HCC group (324.2± 13.38) compared to the LC (229.9± 7.46) and the CHC groups (180.7± 5.27). β-catenin levels were significantly increased ($p<0.001$) in the HCC group (9.58± 0.6) compared to the LC (7.83± 0.5) and the CHC groups (3.96± 0.31). ROC curve analyses were set up for the HCC group with the other groups. The first cut-off value for DKK-1 was 253 pg/ml with sensitivity 88% and specificity 86%. The second one was 301.5 pg/ml with sensitivity 82% and specificity 100%. The cut-off value for β-catenin was 7.35 ng/ml with sensitivity 80% and specificity 74%. A strong positive correlation was observed between DKK-1 and β –catenin (r=0.491). There was also a highly significant positive correlation between DKK-1 and tumor size (r =0.616) as well as between β –catenin and tumor size (r =0.472). Conclusion: DKK-1 and β-catenin may serve as predictors for progression of CHC and LC into HCC.

***Keywords*:** Dickkopf-1, hepatocellular carcinoma, β-catenin, biomarker.

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# List of Abbreviations

| Abb. | | Full Term |
|---|---|---|
| **AAP** | : | aminoantipyrine |
| **AASLD** | : | American association for the study of liver diseases |
| **Ab:** | : | antibody |
| **ADP** | : | adenosine diphosphate |
| **AFP** | : | alpha fetoprotein |
| **AFP-L3** | : | lens culinaris agglutinin fraction of AFP |
| **Ag** | : | antigen |
| **aHSCs** | : | Activated hepatic stellate cells |
| **AIH** | : | auto immune hepatitis |
| **ALB** | : | albumin |
| **ALD** | : | alcoholic liver disease |
| **ALT** | : | alanine aminotransferase |
| **APC** | : | adenomatous polyposis coli |
| **AST** | : | aspartate aminotransferase |
| **ATP** | : | adenosine triphosphate |
| **AUC** | : | area under the curve |
| **Bil** | : | bilirubin |
| **BMI** | : | body mass index |
| **CD $^{4+}$** | : | cluster of differentiation- 4 |
| **CD $^{8+}$** | : | cluster of differentiation- 8 |
| **CHC** | : | chronic hepatitis C |
| **CK1** | : | casein kinase 1 |
| **CRD** | : | cysteine rich domain |
| **CT** | : | computed tomography |
| **Cys-1** | : | amino terminal cysteine rich domains |
| **Cys-2** | : | carboxy terminal cysteine rich domains |
| **DAA** | : | direct acting antiviral agents |
| **DAP** | : | dihydroxy acetone phosphate |
| **DKK1** | : | Dickkopf-1 |
| **E1, E2** | : | envelop protein 1&2 |
| **EASL** | : | European association for the study of the |

| | | |
|---|---|---|
| | | liver |
| **ELISA** | **:** | enzyme linked immunosorbent assay |
| **FDA** | **:** | united states food and drug administration |
| **FN** | **:** | false negative |
| **FP** | **:** | false positive |
| **FZD** | **:** | frizzled receptors |
| **G-3-P** | **:** | glycerol-3-phosphate |
| **Gal** | **:** | galactose |
| **GGT** | **:** | gamma glutamyl transferase |
| **GlcNac** | **:** | N-acetyl-D-glucosamine |
| **GPO** | **:** | glycerol phosphate oxidase |
| **GSK-3** | **:** | glycogen synthase kinase 3 |
| **$H_2O_2$** | **:** | hydrogen peroxide |
| **Hb** | **:** | haemoglobin |
| **HBs Ag** | **:** | hepatitis B surface antigen |
| **HBV** | **:** | hepatitis B virus |
| **HCC** | **:** | hepatocellular carcinoma |
| **HCV** | **:** | hepatitis C virus |
| **HRP** | **:** | horseradish peroxidase |
| **ICC** | **:** | intrahepatic cholangiocarcinoma |
| **IFN-γ** | **:** | interferon gamma |
| **IgG** | **:** | immunoglobulin G |
| **IHC** | **:** | immunohistochemistry |
| **IL-2** | **:** | interleukin -2 |
| **INR** | **:** | international normalized ratio |
| **LC** | **:** | liver cirrhosis |
| **LCA** | **:** | lens culinaris agglutinin |
| **LEF/TCF** | **:** | enhancer factor/T-cell factor |
| **LPL** | **:** | lipase |
| **LRP 5/6** | **:** | low density lipoprotein receptor-related protein 5 and 6 |
| **LT** | **:** | liver transplantation |
| **Man** | **:** | mannose |
| **MIT** | **:** | methyl- isothiazolone |
| **MOH** | **:** | ministry of health |
| **MRI** | **:** | magnetic resonance imaging |

| | | |
|---|---|---|
| **NASH** | : | non alcoholic steatohepatitis |
| **NPV** | : | negative predictive value |
| **NS** | : | non-structural protein |
| **PBC** | : | primary biliary cirrhosis |
| **PBT** | : | proton beam therapy |
| **PEG** | : | poly ethylene glycol |
| **PEIT** | : | percutaneous ethanol injection therapy |
| **PLC** | : | phospholipase-C |
| **PLT** | : | platelet |
| **POD** | : | Peroxidase |
| **PPM1A** | : | Protein phosphatase magnesium-dependent 1A |
| **PPV** | : | positive predictive value |
| **PSC** | : | primary sclerosing cholangitis |
| **qRT-PCR** | : | quantitative real time polymerase chain reaction |
| **RBCs** | : | red blood cells |
| **RBV** | : | ribavirin |
| **RdRP** | : | RNA-dependent RNA polymerase |
| **RFA** | : | radio frequency ablation |
| **ROC** | : | receiver operating characteristic |
| **SBRT** | : | stereotactic body radiotherapy |
| **sFRP** | : | secreted frizzeled related protein |
| **Sia** | : | sialic acid |
| **SVR** | : | sustained viral response |
| **TACE** | : | transarterial chemoembolization |
| **TARE** | : | transarterial radioembolization |
| **TMB** | : | tetramethyl benzidine |
| **TN** | : | true negative |
| **TP** | : | true positive |
| **US** | : | ultrasonography |
| **USA** | : | united states |
| **WHO** | : | world health organization |
| **WIF** | : | WNT inhibitory factor |
| **WNT** | : | wingless related integration site |

# 1- Introduction

Hepatocellular carcinoma (HCC) is the fifth most common neoplasm in men, seventh in women, and third highest cause of cancer-related deaths worldwide **(Atta *et al.*, 2016)**, with 6 million new cases diagnosed annually and approximately 600,000 deaths every year **(Ferlay *et al.*, 2010& Jemal *et al.*, 2011)**. HCC is multifactorial in etiology and complex in the pathogenesis **(Bartlett *et al.*, 2005)**, and usually develops in patients diagnosed with liver cirrhosis **(Masuzaki *et al.*, 2012)**.

Prognosis, survival and management of patients at risk for developing HCC remain challenging in Egypt and worldwide. Furthermore, poor prognosis of patients with symptomatic (HCC) diagnosed clinically at advanced stages suggests an urgent need for new biomarkers detection that can be used for pre-clinical screening for early detection of premalignant lesions and tumors in high risk to hepatitis C infection **(Ghazy *et al.*, 2017).**

The most widely-used HCC biomarker is the serum α-Fetoprotein (AFP). However, the current Western guidelines have excluded AFP measurement for the diagnosis of HCC **(Watany *et al.*, 2017)**, because it has low sensitivity and poor diagnostic yield at the early stage

of HCC (reported sensitivity, 39-64%; specificity, 76-91%; positive predictive value, 9-32%) **(Erdal *et al.*, 2016).**

Lens culinaris agglutinin-reactive AFP (AFP-L3) is the glycosylated subfraction of AFP and is more specific to malignant hepatocytes than AFP. Therefore, it may be useful in distinguishing between elevations in AFP due to benign conditions and HCC **(Bosch *et al.*, 2004)**. However, AFP-L3 elevations were frequently found to be associated with a higher rate of recurrence and a lower survival rate **(Toyodo *et al.*, 2015)**.

HCC has been shown to progress in a multistep manner, although the pathophysiology of this disease remains unclear **(Wang *et al.*, 2017).** There are numerous protein pathways involved in its development and progression, including both stimulatory and inhibitory pathways. Wnt /β-catenin is one pathway that plays a prominent role in HCC **(Whittaker *et al.*, 2010)**. Activation of this pathway clearly contributes to hepatocarcinogenesis as indicated by the detection of recurrent genetic mutations of Wnt/ β-catenin signaling pathway components in HCC that appear especially frequent in HCV-related tumors **(Wang *et al.*, 2017).**

The Dickkopf (DKK) protein family, which has four members (DKK1-4), is a class of secreted Wnt antagonists **(Niehrs *et al.*, 2006)**.

DKK1, a secreted protein, is a known negative regulator of the Wnt signalling pathway, which plays an important role in a variety of cellular processes, including proliferation, differentiation, survival, apoptosis and cell motility**(Zhu *et al.*, 2013).**

DKK1 was frequently found to be overexpressed in patients with Wilms tumor, hepatoblastoma, multiple myeloma and breast cancer. So, DKK-1 was recently reported as a promising biomarker for HCC, even in AFP-negative patients such as the case in chronic liver disease **(Watany *et al.*, 2017)**. The authors addressed this by examining AFP-negative patients and those with early HCC. They convincingly show that raised concentrations of DKK1 in serum could differentiate HCC from chronic hepatitis B (HBV) infection and cirrhosis, and that DKK1 and AFP together improved diagnostic accuracy for HCC versus all controls compared with either test alone **( Zhu *et al.*, 2013).**

As early detection of HCC is essential, new markers with sufficient sensitivity and specificity are needed **(Atta *et al.*, 2016).**