Clinical significance and diagnostic value of serum dickkopf-1 in patients with hepatocellular carcinoma

Yasser M. Fouad, Hala I. Mohamed, Enas M. kamal & Mohamed A. Rasek

To cite this article: Yasser M. Fouad, Hala I. Mohamed, Enas M. kamal & Mohamed A. Rasek (2016) Clinical significance and diagnostic value of serum dickkopf-1 in patients with hepatocellular carcinoma, Scandinavian Journal of Gastroenterology, 51:9, 1133-1137, DOI: 10.3109/00365521.2016.1172337

To link to this article: https://doi.org/10.3109/00365521.2016.1172337

Published online: 10 May 2016.
Clinical significance and diagnostic value of serum dickkopf-1 in patients with hepatocellular carcinoma

Yasser M. Fouada, Hala I. Mohameda, Enas M. kamala and Mohamed A. Rasekb

aDepartment of Tropical Medicine, Minya University, Minya, Egypt; bDepartment of Clinical Pathology, Minya University, Minya, Egypt

ABSTRACT

Background: Hepatocellular carcinoma (HCC) is one of the most prevalent cancers worldwide. It has been widely established that the early detection of HCC enables more treatment options and translates to improved survival.

Aim: To assess the diagnostic accuracy of DKK1 as a serum protein marker for HCC by examining its diagnostic sensitivity and specificity in HCC.

Methods: We analyzed data for 50 patients with hepatitis C virus (HCV) related HCC as the studied group. Twenty patients with chronic hepatitis C and 20 patients with HCV-related liver cirrhosis will serve as control group. DKK1 was measured in serum by ELISA. We used receiver operating characteristics (ROC) to calculate its diagnostic accuracy.

Results: We assessed serum DKK1 in 90 participants: 50 with HCC (studied group), 20 with chronic HCV infection, and 20 with liver cirrhosis (as control group). Serum concentration of DKK1 was significantly higher in HCC group and values did not differ significantly between the two control groups. We performed multivariate regression analysis using AFP level, number of focal lesions, focal lesion size and Portal vein thrombosis as an independent variable. ROC curves showed the optimum diagnostic cut off was 1.5 ng/mL (sensitivity 67.5%, specificity 89.3%).

Conclusion: Serum DKK1 could potentially be used for early diagnosis of HCC and complement measurement of AFP in the diagnosis of HCC.

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common malignant disease and the third leading cause of cancer-related death worldwide.[1] Across all the countries, 5-year overall survival is only 3–5%.[2] This dismal outcome is partly due to the lack of an effective method for timely diagnosis, which leads to only 30–40% of the patients with HCC being suitable for potentially curative treatments at the time of diagnosis.[3] Alpha-fetoprotein (AFP) is the best serum biomarker for the diagnosis of HCC, but sensitivity is low (25–65%) at the commonly used cut off of 20 ng/mL, particularly in detection of early-stage HCC.[4]

DKK1, a secreted protein, is known as a negative regulator of the Wnt signaling pathway. The Wnt pathway plays an important role in development and in regulating adult stem cell systems. A variety of cellular processes are mediated by Wnt signaling, including (proliferation, differentiation, survival, apoptosis, and cell motility). Loss of regulation of these pathways can lead to tumorigenesis, and the Wnt pathway has been implicated in the development of several types of cancers.[5]

The activity of the Wnt family is antagonized by several secreted factors including DKK. Other studies have shown overproduction of DKK1 in patients with Wilms tumor, hepatoblastoma, hepatocellular carcinoma, breast cancer, and multiple myeloma, indicating that DKK1 has a potential oncogenic role in these tumors rather than acting as a tumor suppressor through inhibition of Wnt signaling.[6]

Yu and colleagues showed upregulated DKK1 in HCC tissues on microarray analysis and suggested it had prognostic value for HCC, especially in patients with early-stage disease or AFP-negative status.[7]

Tung and coworkers calculated sensitivity of 34% and specificity of 100% for DKK1 in the diagnosis of HCC.[8] The aim of this study was to assess the diagnostic accuracy of DKK1 as a serum protein marker for HCC by examining its diagnostic sensitivity and specificity in HCC.

Patients and methods

A total of 50 patients with recently diagnosed HCC (studied group) and 20 chronic hepatitis patients as well as 20 cirrhotic patients (control group) were enrolled in the present study. Clinical and pathological data were collected from patient records and pathology reports. The study was approved by the institutional review board of the Minia Faculty of Medicine, Minya University, Egypt. Written informed consent was obtained from every participant (for both participation in the study and publication of the data).

HCC was defined on the basis of ultrasound, CT, or MRI characteristics and biochemistry (AFP serology and liver function enzymes). The diagnosis of cirrhosis and chronic hepatitis was based on histopathology of liver biopsy.
Fasting blood was taken for all participants and serum was collected and stored at −80 °C. Serum DKK-1 levels were measured by enzyme-linked immunosorbent assay (ELISA) with immunoassay kit (Miltenyi, Gladbach, Germany) according to the manufacturer’s directions. The level of protein was obtained through standard curve. Results were reported as concentration of DKK1 ng/ml in samples.

**Statistical analysis**

Statistical analyses were performed using SPSS 13.0 and Graph Pad Prism 5 (Graph Pad Software Inc., La Jolla, CA). Numerical variables were recorded as means ± SD and analyzed by independent tests. Categorical variables were presented as rates and analyzed by using the chi-square test or Fisher’s exact test software. The correlation between DKK1 concentrations in serum and focal lesion sizes was analyzed with Spearman’s test.

Multivariate Regression Analysis models were performed in SPSS and ROC curve analysis was performed for area under the curve (AUC) values for the combined markers (DKK-1 and AFP).

**Results**

Fifty patients with HCC were prospectively enrolled in the present study. Forty patients with liver cirrhosis and chronic hepatitis were served as control group. All the patients were HCV positive. Mean serum DKK-1 level was 16.8 ± 2.9 ng/ml in HCC; 2.9 ± 0.99 in chronic HCV and 3.5 ± 0.95 in LC group. AFP level was 284.2 ± 263.8 in HCC; 53.2 ± 13.2 in CHC and 91.7 ± 16.7 in LC. Patients’ characteristics are summarized in Table 1.

As shown in Figure 1, serum DKK-1 levels were significantly high in HCC patients than liver cirrhosis group (<0.05) but there was no significant difference with chronic HCV group (<0.3).

Regarding to the relation between tumor size and DKK1 biomarkers, Serum DKK1 correlates with tumor size in advanced hepatocellular carcinoma (diameter ≥5 cm) (Figure 2). However, this correlation is not evident in hepatocellular carcinoma smaller than 3 cm in diameter.

To investigate the imaging, laboratory factors which may affect the measured value of DKK1, we performed multivariate regression analysis using AFP level, number of focal lesions, focal lesion size and Portal vein thrombosis as an independent variable. We found that DKK1 significantly associated only with multiple focal lesions, focal lesion size >5 cm and Portal vein thrombosis (p < 0.03; p < 0.001; p < 0.002; respectively) (Table 2).

ROC curves showed the optimum diagnostic cut off for DKK1 was 1.53 ng/mL (AUC 0.824; sensitivity 67.5%, specificity 89.3%) (Figure 3 (Table A)). The optimum cut off value for AFP was 15.4 ng/mL (AUC 0.789; sensitivity 65.4%, specificity 76.7%).

**Discussion**

Egypt has the highest prevalence of adult HCV infection in the world (15–25%).[9] About 85% of these develop persistent infection and are at risk of long-term complications like liver cirrhosis and HCC.[10] To date, the diagnostic role of serum AFP in advanced HCC is well recognized. However, a serum AFP level >400 ng/mL was found in only 2.4–22% of the patients with small HCC (tumor size <3 cm).[11]

Our results showed that the levels of serum DKK-1 were significantly increased in patients with HCC compared with LC group. Furthermore, we found that serum DKK-1 level expression levels were significantly positively correlated with focal lesion sizes, suggesting that DKK-1 might be involved in the carcinogenesis and metastasis of HCC.

This is in agreement to Shen and coworkers who found that levels of DKK1 in serum were significantly higher in patients with HCC than in all controls. That can be explained by the assuming that DKK1 might act as a tumor suppressor and that is why it increased in their study.[12]

As a result, serum DKK1 level correlated with tumor size, consequently, patients with bigger tumors tend to have higher levels of DKK1.

In addition, we found that the serum level of DKK-1 in HCC patients and chronic HCV groups had no significant difference, this is similar to what was found by Shen and coworkers.[12] This may be because of the activity of HCV virus which leads to accumulation of β-catenin in the cytoplasm.
and nucleus with subsequent transcriptional activation of Wnt pathway targets.[13]

DKK1 silenced in cancer owing to methylation of DKK there was an inverse relation between expressions DKK-1 and DKK-1 methylation. Loss of DKKs may facilitate tumor genesis through β-catenin/T-cell factor – independent mechanism.[14]

Since DKK-1 is a secreted protein, serum level of DKK-1 and its prognostic potential have been investigated in several cancers. For example, Yang H et al. found that the mean serum level of DKK-1 in patients with early hepatocellular carcinoma was significantly higher than that in patients with cirrhosis, non-cirrhotic chronic hepatitis B, benign liver tumors and healthy individuals. The patients with a high serum DKK-1 level had a poorer overall survival and relapse-free survival than those with a low expression level.[12]

In the assessment of diagnostic accuracy, the serum DKK-1 had greater AUC, sensitivity and specificity value than did AFP in patients with HCC. Previous cross sectional ROC studies of AFP as a screening tool (at various cutoff values) have shown sensitivities between 25 and 65%, and specificities between 79% and 95%.[15]

Shen et al. reported that The ROC analysis gave an optimal cutoff value of 2.153 ng/mL for DKK1, with a sensitivity of 69.1% and specificity of 90.6%. They concluded that serum DKK1, alone or in combination with AFP, was better than AFP alone in the diagnosis of HCC.[12]

Multivariate regression analysis further confirmed that DKK-1 was significantly associated with multiple focal lesions, portal vein thrombosis, and large tumor size. Previous data showed that high level of DKK1 expression in HCC tissues correlated with poor prognosis of HCC patients, and that DKK1 was an independent prognostic factor for overall survival and diseases-free survival in HCC patients.[16]

**Conclusion**

Our results indicate that serum DKK1 could potentially be used to diagnose HCC and complement measurement of AFP in the diagnosis of HCC.

HCC: hepatocellular carcinoma; LC: liver cirrhosis; CHC: chronic hepatitis C

![Figure 1](image1.png)

**Figure 1.** Serum DKK-1 levels in HCC patients versus LC and chronic hepatitis C.

![Figure 2](image2.png)

**Figure 2.** Correlation between focal lesion size and serum DKK1 level.

**Table 2.** Multiple linear regression analysis using DKK1 as dependent variable.

<table>
<thead>
<tr>
<th>OR (95% CI)</th>
<th>*p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AFP</strong></td>
<td></td>
</tr>
<tr>
<td>&gt;400</td>
<td>0.6 (0.55–0.94)</td>
</tr>
<tr>
<td>&lt;400</td>
<td>1</td>
</tr>
<tr>
<td><strong>Number of tumor</strong></td>
<td></td>
</tr>
<tr>
<td>Multiple</td>
<td>1.8 (0.28–3.03)</td>
</tr>
<tr>
<td>Single</td>
<td>1</td>
</tr>
<tr>
<td><strong>Tumor size</strong></td>
<td></td>
</tr>
<tr>
<td>&gt;5ml</td>
<td>3.1 (1.31–7.52)</td>
</tr>
<tr>
<td>&lt;5ml</td>
<td>1</td>
</tr>
<tr>
<td><strong>Portal vein thrombosis</strong></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>2.8 (1.29–5.85)</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
</tr>
</tbody>
</table>

OR (95% CI): Odds ratio (95% confidence interval); AFP: Alpha Feto protein. *Statistically significant.
Acknowledgements

The authors would like to express sincere appreciation to Dr. Madeha Makhlouf for her valuable advice for the preparation of the study. The authors also thank Dr. Yasser Mahrous for giving the access to the research facilities and for his insightful comments and encouragement. All human participants involved in this study were in accordance with the ethical standards of Clinical Research Committee of Minya University Hospital. Written informed consent was obtained from Clinical Research Ethics Committee of Minya University Hospital.

Disclosure statement

The authors declare that they have no competing interests.

Funding information

All authors declare that there was no fund provided to this research regarding to study design; in the collection, analysis, and interpretation of data; in the writing of the manuscript. This study had not been funded by any agency.

References

[8] Tung EK, Mak CK, Fatima S, et al. Clinicopathological and prognostic significance of serum and tissue Dickkopf-1 levels in human hepatoce...


学霸图书馆
www.xuebalib.com

本文献由“学霸图书馆-文献云下载”收集自网络，仅供学习交流使用。

学霸图书馆（www.xuebalib.com）是一个“整合众多图书馆数据库资源，
提供一站式文献检索和下载服务”的24小时在线不限IP图书馆。
图书馆致力于便利、促进学习与科研，提供最强文献下载服务。

图书馆导航：
图书馆首页 文献云下载 图书馆入口 外文数据库大全 疑难文献辅助工具