Evaluation of serum dickkopf-1 as a tumor biomarker for diagnosis and prognosis of hepatocellular carcinoma in patients with cirrhotic liver

Fatma Allam Abd-Elal, Galal Eldin Moustafa Elkassas, Gamal Kamel Kasem, Mohamed Yousef Rabea and Sara Amr Hamam

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Abstract

Background: Liver cancer is the fifth most common cancer and the second most frequent cause of cancer-related death globally. Diagnosis of hepatocellular carcinoma (HCC) should occur in an early stage, so that the patient benefits from earlier diagnosis, through treatment using established algorithms. The research aimed to evaluate the significance of Dickkopf-1 (DKK1) as a tumor biomarker for the diagnosis and prognosis of HCC in cirrhotic cases.

Methods: This prospective, randomized, controlled research was carried out on 120 individuals who were classified as follow: Group I: comprised 40 cases with cirrhotic liver and HCC. Group II: comprised 30 cases with cirrhotic liver without HCC. Group III: comprised 30 cases with chronic hepatitis without cirrhosis. Control group: comprised 20 healthy individuals. Serum DKK-1 level were measured to all participants but for HCC patient group it was measured before intervention and one month after intervention (with the first CT after intervention).

Results: Six cases of group I underwent microwave ablation, 13 cases underwent RFA, 20 cases underwent trans-arterial chemotheraphy (TACE) and one patient underwent liver transplantation. Thirteen cases of group I were well ablated following loco regional therapy and no recurrence or de novo lesions appeared during follow up. Residual activity or de novo lesions or recurrence appeared in 26 cases who required second cession of ablation. alpha-fetoprotein (AFP) in group I was ranging between 3.6 to 2400 ng/ml with mean 698.870 ng/ml. It was higher in group I than groups II, III, and IV. DKK1 level was significantly higher in group I than groups II, III and IV, also, was significantly higher in group II than groups III and IV and it was significantly higher in group III than group IV.

Conclusions: Serum DKK1 could serve as a potential diagnostic biobiomarker for HCC. DKK1 might be utilised as a predictor of therapeutic ablation outcome in cases with hepatocellular carcinoma.

Keywords: Serum dickkopf-1, hepatocellular carcinoma, cirrhotic liver

Introduction

Hepatocellular carcinoma (HCC) is the most common type of liver cancer. Globally, it is the fifth 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 alpha-fetoprotein (AFP) measurement and ultrasound every 6–12 months as a screening strategy for HCC in high-risk cases [3].

AFP is the current biomarker 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 cirrhotic liver (LC). (b) Low sensitivity as AFP level may be normal in 40% of HCC cases. 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 biobiomarkers 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 cases with HCC. Qi et al. reported that HCC cases had a higher serum DKK-1 level compared with the controls and non-HCC liver disease cases [10].

**Patients and Methods**

This prospective cohort research was performed on 120 individuals from the outpatient clinics and incases of Tropical Medicine and Infectious Diseases Department at Tanta University Hospitals in the duration from October 2019 to October 2021. Individuals were divided into four groups:

- **Group I**: comprised 40 cases with cirrhotic liver and HCC
- **Group II**: comprised 30 cases with cirrhotic liver without HCC
- **Group III**: comprised 30 cases with chronic hepatitis without cirrhosis.
- **Control group**: comprised 20 healthy individuals age and sex matched with the participants.

**A. Inclusion criteria**

- Cases with HCC with cirrhosis within the criteria of treatment.
- Cases with cirrhosis without HCC.
- Patient with chronic hepatitis without cirrhosis.

**B. Exclusion criteria**

- Cases with malignancies other than HCC.
- Previously treated HCC cases.
- Child C (HCC).

**Methodology**

All cases and controls were subjected to the following:

- Full history taking.
- Clinical examination.
- Laboratory investigations:
  1. Complete blood count, prothrombin time, serum creatinine & urea, serum Aspartate aminotransferase, serum Bilirubin, serum albumin, serum AFP were measured to all participants.
  2. Serum DKK-1 level were measured to all participants by human DKK-1 enzyme-linked immunosorbent assay kit according to the manufacturer’s instructions once. But for HCC patient group it was measured before intervention and one month after intervention (with the first CT after intervention).

**Radiological examination**

- Abdominal ultrasonography was done to assess the presence of cirrhotic liver, ascites, and hepatic focal lesions.
- Triphasic computed tomography scan:

HCC was diagnosed based on the existence of a characteristic vascular pattern consisting of early arterial enhancement, quick washout of portal venous phases, and a delayed phase.

**Results**

<table>
<thead>
<tr>
<th>History</th>
<th>Groups</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>41-66</td>
<td></td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>57.975±5.475</td>
<td>9.555±3.767±8.195±9.718</td>
</tr>
<tr>
<td>Chi-Square</td>
<td>N % N % N % N %</td>
<td>N % X²</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>24</td>
<td>21</td>
</tr>
<tr>
<td>Female</td>
<td>16</td>
<td>9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>History</th>
<th>Groups</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAAS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>25.0%</td>
<td>96.67%</td>
</tr>
<tr>
<td>Yes</td>
<td>75.0%</td>
<td>3.33%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Examination</th>
<th>Groups</th>
<th>Chi-Square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascites</td>
<td>N % N %</td>
<td>63.529 &lt;0.001*</td>
</tr>
<tr>
<td>LL. oedema</td>
<td>N % N %</td>
<td>42.065 &lt;0.001*</td>
</tr>
<tr>
<td>HE</td>
<td>N % N %</td>
<td>25.714 &lt;0.001*</td>
</tr>
</tbody>
</table>

*Significant difference, LL. Oedema: lower limb oedema, HE: hepatic encephalopathy.
As regard liver size, there was significant increase in liver size in group I than groups II, III and IV (p<0.001). As regard cirrhosis, all cases in groups I and II had cirrhotic liver, while those in group III and IV had non cirrhotic liver (p<0.001). Regarding splenomegaly, there was significant increase of splenic size in group I than groups II, III and IV (p<0.001) and significant increase in group II than groups III and IV (p<0.001). As regard splenectomy, there was significant increase of splenectomy in group II than groups I, III and IV (P= 0.006).

As regard ascites, there was significant increase of ascites in group II than groups I, III and IV (p<0.001) (table 5).

Table 6: Comparison between the four groups as regard serum DKK1

<table>
<thead>
<tr>
<th>DKK1</th>
<th>Groups</th>
<th>Kruskal-Wallis Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group I</td>
<td>Group II</td>
</tr>
<tr>
<td>Range</td>
<td>108.026 [-]</td>
<td>692.76</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>189.945(180.25-213.553)</td>
<td>155(133.928-191.338)</td>
</tr>
<tr>
<td>Mann-Whitney Test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I&amp;II</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

*Significant difference, DKK1= dekkopf-1

Table 7: Assessment of prognostic measure of DKK1 in follow up after ablation

<table>
<thead>
<tr>
<th>DKK1</th>
<th>Hepatic FL Triphasic CT findings After</th>
<th>Mann-Whitney Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Well ablated lesions</td>
<td>Residuals + New FL</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
</tr>
<tr>
<td>Before</td>
<td>180.34 (128.24-216.21)</td>
<td>189.95 (183.52-208.84)</td>
</tr>
<tr>
<td>After</td>
<td>98.09 (73.02-188.08)</td>
<td>201.07 (186.65-233.65)</td>
</tr>
<tr>
<td>Wilcoxon Signed Ranks Test</td>
<td>0.001*</td>
<td>0.033*</td>
</tr>
</tbody>
</table>

*Significant difference, W&R= Wilcoxon & Ranks
DKK1 level was significantly decreasing in the follow up periods after ablation in well ablated lesions, but not significantly decreasing in the follow up period in residuals plus new lesions (P=0.001 & 0.343 respectively), but it was significantly increasing in residuals without new lesion P=0.033 (table 7).

**Table 8: Correlation between clinical history and serum DKK1 level**

<table>
<thead>
<tr>
<th>Correlations</th>
<th>Male</th>
<th>Mann-Whitney Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Median (IQR)</td>
</tr>
<tr>
<td>Sex</td>
<td>63</td>
<td>166.19(136.39-196.96)</td>
</tr>
<tr>
<td>Smoking</td>
<td>37</td>
<td>179.60(125.48-190.55)</td>
</tr>
<tr>
<td>Abd. Pain</td>
<td>62</td>
<td>161.78(127.93-189.91)</td>
</tr>
<tr>
<td>DM</td>
<td>38</td>
<td>181.19 (144-213.57)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>57</td>
<td>148.98 (115.23-183.37)</td>
</tr>
<tr>
<td>DAAS</td>
<td>43</td>
<td>189.05 (157.36-210.91)</td>
</tr>
<tr>
<td>Hepatitis virus biomarkers</td>
<td>76</td>
<td>167.44 (122.68-191.74)</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>180.04 (139.24-213.55)</td>
</tr>
</tbody>
</table>

* = Significance, DM= diabetes mellites, DAAS= Direct acting antivirals

No significant correlation was found between sex, DM, hypertension or Child Pugh and the serum level of DKK1 (P>0.05). There was a significant positive correlation between serum level of DKK1 and smoking, abdominal pain, history of treatment with DAAS and chronic viral hepatitis C (p=0.033, <0.001, 0.005 and <0.001) respectively (table 8).

**Table 9: AFP and DKK1 level**

<table>
<thead>
<tr>
<th>Correlations</th>
<th>DKK1 Before</th>
<th>Mann-Whitney Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Median (IQR)</td>
</tr>
<tr>
<td>Smoking</td>
<td>63</td>
<td>166.19(136.39-196.96)</td>
</tr>
<tr>
<td>Age</td>
<td>37</td>
<td>179.60 (125.48-190.55)</td>
</tr>
<tr>
<td>HB%</td>
<td>62</td>
<td>161.78 (127.93-189.91)</td>
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<tr>
<td>TLC</td>
<td>38</td>
<td>181.19 (144-213.57)</td>
</tr>
<tr>
<td>Platelets</td>
<td>57</td>
<td>148.98 (115.23-183.37)</td>
</tr>
<tr>
<td>RBCs</td>
<td>43</td>
<td>189.05 (157.36-210.91)</td>
</tr>
<tr>
<td>ALT</td>
<td>76</td>
<td>167.44 (122.68-191.74)</td>
</tr>
<tr>
<td>AST</td>
<td>24</td>
<td>180.04 (139.24-213.55)</td>
</tr>
</tbody>
</table>

* = Significance

**Table 10: ROC curve of DKK1 between Cases and Control**

<table>
<thead>
<tr>
<th>ROC curve between Cases and Control</th>
<th>Cutoff</th>
<th>Sensitivity</th>
<th>Specificity.</th>
<th>PPV</th>
<th>NPV</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>DKK1</td>
<td>&gt;107.19</td>
<td>96.0</td>
<td>80.0</td>
<td>96.0</td>
<td>80.0</td>
<td>0.932</td>
</tr>
</tbody>
</table>

Serum DKK1 at cut-off >107.19 Pgm/l can differentiate between group I (HCC group), group II (cirrhotic group), group III (noncirrhotic group) and group IV (control group) with 96.0% sensitivity, 80% specificity, 96.0% PPV, 80% NPV and 0.932 AUC (table 10).

**Discussion**

In this research, HCC commonly presented in males (24 males) more than females (16 females). This agreed with Lee et al., (2015) and Liu et al., (2017) who pronounced that, men are at a higher risk of HCC compared with women especially a young woman per se because of protective effect of estrogen which inhibits inflammatory responses, prevents oxidative stress, and induces apoptotic cell death (El Mahdy et al., 2016), and low incidence of risk factors however the potential molecular mechanisms remain to be elucidated (Li et al., 2012). In addition, Wu et al. (2018) identified a male preponderance among HCC cases and suggested that gender-specific variations in exposure to risk variables may account for the higher prevalence of liver cancer in men [11-15]. This gender difference can be explained by biological and environmental factors. As revealed by Naugler et al., the oestrogen hormone level partially contributes to the reduction of interleukin (IL-6)-mediated inflammation, which decreases both compensatory proliferation and liver damage (2007). According to Ma et al., testosterone in
males can boost the signalling of androgen receptors, which promotes liver cell growth (2014). In addition, Abd-Elsalam et al. (2018) reported that Male exposure to liver carcinogens, such as occupational exposure to chemicals, alcohol, and smoking, as well as hepatic viral infection, is higher than female exposure, which explains the HCC incidence disparity [16-19].

75% of the HCC group had history of treating chronic hepatitis C (CHC) viral infection with DAAS and achieving SVR. This agreed with Reig et al., (2016), Conti et al., (2016), Ravi et al., (2017) and Piero et al., (2019) who first hypothesised that DAAs could enhance early de novo HCC development or relapse. Also, our finding contradicted the prospective North Italian research by Romano et al. (2018). It noted that the risk for HCC recurrence following DAA treatment reduces gradually with time after SVR, indicating that early HCC incidence after SVR may be attributable to the pre-existence of undetected tiny tumors that may expand into multinodular or infiltrating tumors after DAA [19-24].

This is supported by Kumar et al., (2014) who concluded that right upper quadrant abdominal pain is one of the most frequently reported symptoms for cases with HCC, and pain can be parietal or visceral as well; 66.67% of cases in group (II) had abdominal pain, which is supported by Rogal et al., (2015) who discovered that pain has been found in up to 82% of cases with cirrhosis [25-26].

As regards DM, there was a substantial increase in group (I); 37.5% of cases in group I had DM. This was consistent with El-Serag et al. (2006), ’s meta-analysis of 13 cohort studies and 13 case-control studies, which revealed that DM is linked with a 2.5-fold higher risk of HCC [27].

Group (II) also, showed significant decrease in Hb% and platelet count which are complications of cirrhosis as proved by (Qamar et al., 2009). Also, Basili et al., (2019) and Zanetto et al., (2021) concluded that cases with cirrhosis have profound alterations of primary hemostasis that include low platelet count, and complex alterations of platelet function, this significant increase of ascites, lower limb oedema, hepatic encephalopathy, low hemoglobin and low platelet count in group was because this group involved 30 cirrhotic cases with different Child pugh score A, B and C while group I (HCC cases) all were Child A to be candidate for treatment and group III involved non cirrhotic cases.

In our research, cases in groups I and II (HCC and cirrhotic liver) had a significant increase in serum AST and ALT, total and direct bilirubin and INR, while patient in group III (chronic hepatitis without cirrhosis) had a significantly higher platelet count and albumin concentration, P. activity and ALT, these results are in agreement with (Zekri et al., 2011 and Mohamed et al., 2020) [28-32].

In our research cases in group II (cirrhosis) were significantly higher than groups I, III and IV as regard serum urea and creatinine level, this finding was in agreement with Llach et al., (1988) who concluded that renal dysfunction is a major complication that accompanies cirrhosis and is associated with poor prognosis, and Serra et al., (2004) who described that Serum creatinine (Cr) is increasingly being integrated into predictive models for cases of cirrhotic liver in failure [33-34].

Of the three patient groups, I, II and III there was significant higher liver fibrosis as measured by fibroscan in group I than groups II and III and significant higher liver fibrosis in group II than group III this agreed with Ebrahim et al., (2020) They stated that fibroscan can be an effective method for detecting HCC in high-risk cirrhotic cases and that including fibroscan into the present HCC screening routine in hepatitis C cirrhotic cases can be of considerable benefit [35].

Thankfully, tumor biomarkers with a high degree of specificity and sensitivity can detect the existence of the majority of human malignancies. AFP is the most prevalent tumor biomarker utilized in HCC screening (Yi et al., 2013) [36].

On comparing the four studied groups as regard the alpha-fetoprotein (AFP), its level was significantly higher in group I than group II, III and IV which showed that AFP level can distinguish HCC cases from cirrhosis cases, HCC cases from chronic viral hepatitis without cirrhosis cases and HCC cases from controls. This result agreed with Erdal et al., (2016) and Younis et al., (2019) who showed that AFP level can distinguish HCC cases from cirrhosis cases, and HCC cases from controls [37-38].

There was a significant correlation between smoking and serum level of DKK1 which agreed with Jorde et al., (2019) who concluded that smokers had significantly higher DKK1 than non-smokers [39].

In this work there was a significant association between serum DKK1 and hepatitis C which agreed with Eldeeb et al., (2020) who described significant increase in serum DKK-1 level in HCV cirrhotic cases with HCC than HCV cirrhotic cases without HCC. It may explain why DKK-1 may function as a tumor suppressor. In this work six cases were managed by microwave ablation, thirteen cases were managed by radiofrequency ablation (RFA), twenty-two cases underwent TACE and one patient underwent liver transplantation. Microwave (MWA) maneuver was preferred for lesions near great vessel to avoid heat effect [40].

All cases managed through TACE had residual activity or de novo lesions and require more sessions. This result agreed with Pomfret et al., (2010) who concluded that About 64% of cases were submitted to second TACE, while only few cases (26%) were submitted to third TACE using an “on demand” policy [41].

In the current research AFP level was significantly decreasing in the follow up period after ablation in cases with well ablated lesions (P=0.001). It was also, decreasing in cases with residual activity in their lesions but insignificant decrease (P=0.654), while, it was significantly increasing in the follow up period after intervention in cases with de novo lesions, which supports the prognostic role of AFP after therapeutic HCC intervention and agreed with Hakeem et al., (2012) who reported that there is a significant correlation between AFP and HCC prognosis, Imamura et al., (2003) who stated that persistent fluctuations in AFP level may be a predictive factor for HCC development, and AFP is the most often tested indication for detecting HCC relapse [42-43].

In this research, DKK-1 level was significantly decreasing in the follow up periods after ablation in well ablated lesions, which was in agreement Sharaf et al., (2016) research, whose serum DKK1 level decreased following radiofrequency ablation or alcohol injection of HCC. In addition, Tung et al. (2011) reported the lowering of serum DKK1 level in HCC cases following liver resection. Therefore, elevated DKK1 may be the result of its overproduction by tumor cells [44-45].
Kim et al. (2015) reported that the DKK-1 cutoff value was 1.01 ng/mL (AUC=0.829; sensitivity 90.7%, specificity 62%), but the AFP cutoff value was 7.50 ng/mL (AUC=0.794; sensitivity 69.3%, specificity 87.7%) [46]. Kim et al. (2006) determined that the diagnostic sensitivity of AFP as a serum biomarker for the identification of HCC with cut-off level between 20 and 100 ng/ml is around 47.3%. This disparity may be due to changes in tumor size, cirrhosis aetiology, or AFP assay technique [21]. In a Chinese research conducted by Chan et al. (2014), the best AFP cut-off measure for the diagnosis of HCC was determined to be 200 ng/mL, with a sensitivity of 47.7% and a specificity of 97.7%.

DKK1 level may be useful as a diagnostic biomarker for HCC and treatment methods outcome monitoring, particularly in instances with average AFP, and it may enhance the sensitivity of AFP when paired with it [60].

Conclusions
The present research suggests that serum DKK1 might act as a possible bio biomarker for the diagnosis of HCC. DKK1 might be utilized as a predictor of therapeutic ablation success in cases with hepatocellular carcinoma.

Conflict of Interest
Not available

Financial Support
Not available

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