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# Combination of DKK1 and AFP improves diagnostic accuracy of hepatocellular carcinoma compared with either marker alone

**LIVER**

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Original Article

## ABSTRACT

**Background/Aims:** The Wnt/ß-catenin pathway plays a prominent role in hepatocellular carcinoma (HCC). The Dickkopf (DKK) proteins (DKK1-4) are known Wnt antagonists; the overexpression of DKK1 has been demonstrated in HCC, and increased DKK3 methylation in the HCC tissue is associated with worse prognosis. Thus, the aim of our study was to demonstrate the diagnostic accuracy of serum DKK1 and DKK3 in HCC in comparison with that of serum alpha-fetoprotein (AFP).

**Materials and Methods:** We included consecutive 40 HCC patients, 54 cirrhosis patients, and 39 healthy controls. Serum DKK1 and DKK3 levels were measured by an enzyme-linked immunosorbent assay, and serum AFP levels were measured by a chemiluminescence assay.

**Results:** The AFP levels differed in each group and could help differentiate between groups ($p<0.001$). The DKK1 levels could help differentiate the HCC group from cirrhosis and control groups ($p<0.001$), and the DKK3 levels could help differentiate HCC and cirrhosis groups from the control group ($p<0.001$). Combined usage of DKK1 and AFP increased the diagnostic yield, with a sensitivity, specificity, positive predictive value, and negative predictive value of 87.5%, 92.3%, 92.1%, and 87.8%, respectively.

**Conclusion:** Although AFP is superior to DKK1 and DKK3 in the diagnosis of HCC, the combination of DKK1 and AFP showed a better diagnostic yield than AFP alone.

**Keywords:** Alpha-fetoprotein, diagnosis, Dickkopf proteins, hepatocellular carcinoma, biomarker

## INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most frequent cancer and the third leading cause of cancer-related deaths worldwide (1). Cirrhosis due to chronic hepatitis B and C infection is the most common cause of HCC (2). Imaging methods are insufficient to diagnose the disease at an early stage, and the poor prognosis of HCC can be largely attributed to delayed diagnosis, with 5-year survival rates for Tumor Node Metastasis (TNM) stages I and III HCC being 55% and 16%, respectively (3).

Alpha-fetoprotein (AFP), which is widely used in clinical practice, 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%) (4,5). Accordingly, new markers, which have higher sensitivity and specificity, are needed to improve the diagnosis at the early stage and potentially increase the survival.

Hepatocellular carcinoma has been shown to progress in a multistep manner, although the pathophysiology of this disease remains unclear. There are numerous protein pathways involved in its development and progression, including both stimulatory and inhibitory pathways. Wnt/ß-catenin is one pathway that plays a prominent role in HCC (6). The Dickkopf (DKK) protein family, which has four members (DKK1–4), is a class of secreted Wnt antagonists (7). These proteins consist of

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225–350 amino acids; the molecular weights of DKK1, DKK2, and DKK4 range between 25 and 29 kDa, while that of DKK3 is slightly higher at 38 kDa. While DKK1, DKK2, and DKK4 have been shown to bind to the Wnt co-receptor LRP5/6 and block the formation of the FZD–Wnt–LRP5/6 complex (8), the role of DKK3 in inhibiting Wnt signaling remains unclear.

The overexpression of DKK1 in the HCC tissue was first reported by Yu et al. (9), who demonstrated the correlation between the overexpression of DKK1 and nuclear/cytoplasmic ß-catenin accumulation in the HCC tissue. Moreover, a negative feedback mechanism of DKK1 to inhibit TCF-mediated transcription and nuclear/cytoplasmic ß-catenin accumulation in HCC cell lines was demonstrated, whereas this was not detectable in a non-cancer liver tissue.

DKK3 methylation is higher in tissues of HCC patients than in tissues of cirrhosis patients without HCC. Furthermore, high DKK3 methylation levels are associated with poor prognosis in HCC (10).

Based on these previous studies, we hypothesized that serum DKK1 levels are increased in HCC patients because of their overexpression in the HCC tissue. In addition, we aimed to demonstrate the diagnostic efficacies of serum levels of DKK1 and DKK3 in the early stages of HCC and compared them with that of AFP.

## MATERIALS AND METHODS

### Study design

In total, 133 subjects were enrolled in our study and were divided into three groups: HCC (n=40), cirrhosis (n=54), and control (n=39) groups. All subjects were sequentially enrolled in Gazi University School of Medicine, Department of Gastroenterology. The diagnosis of 22 HCC patients was made by histopathology. If histopathology was not present, the diagnosis of HCC was based on the American Association for the Study of Liver Diseases (AASLD) guidelines (11) and confirmed by imaging modalities (ultrasound, magnetic resonance imaging, or computed tomography) and biochemistry (AFP and liver function test). Patients with another malignancy were excluded from the study. Early stage and very early stage were classified according to the Barcelona Clinic Liver Cancer (BCLC) staging system. We defined BCLC stage A as early-stage HCC and BCLC stage 0 as very-early-stage HCC.

Cirrhosis was diagnosed on the basis of liver histopathology or imaging, clinical, and laboratory findings of portal hypertension. The control group consisted of healthy subjects without risk factors for viral hepatitis, history of liver disease, or alcohol consumption. All subjects in the control group were documented to have normal liver biochemical test results.

Informed consent was obtained from each patient included in the study. The study was approved by the local ethics committee. In total, 10 mL blood was collected from each subject, and the serum was stored at –80°C until analysis. AFP was measured by the chemiluminescence method (ARCHITECH system; Abbott Laboratories, Abbott Park; IL, USA). The upper limit of the normal level is 7 ng/mL. DKK1 and DKK3 were measured using a commercially available enzyme-linked immunosorbent assay kit (RayBio®, RayBiotech, Inc.; Norcross, GA, USA). The performance characteristics of the human DKK1 and DKK3 enzyme-linked immunosorbent assay kits were as follows: intra-assay coefficient of variation, 9; inter-assay coefficient of variation, 11.

### Statistical analysis

SPSS version 15.0 (SPSS Inc.; Chicago, IL, USA) was used in statistical analysis. Data are presented as x±y, where x is the mean and y is the standard deviation. One-way ANOVA was used to test differences between the three independent (HCC, cirrhosis, and control) groups. The respective specificities, sensitivities, and areas under the curves were assessed on the basis of 95% confidence intervals (CIs) and by constructing receiver operating characteristics (ROC) curves. The optimum cutoff values were determined by first maximizing the sum of the sensitivity and specificity and then minimizing the overall error (E), as calculated by $E=\sqrt{\sum(1-\text{sensitivity})^2 + 1 - \text{specificity})^2}$. The diagnostic accuracy of combinations of AFP, DKK1, and DKK3 was calculated by estimating the functions of the combined marker by binary logistic regression. The values of these functions were used as one marker and subjected to ROC analysis (Table 1) (12). The following model was used as a basis for sample calculation of a new variable for the combination of DKK1, DKK3, and AFP:

$$\text{Log } [p/(1-p)]=b_0+(b_1\times\text{DKK1})+(b_2\times\text{DKK3})+(b_3\times\text{AFP})$$

A new variable-predicted probability (p) for HCC was created using an equation obtained by binary logistic regression (all HCC versus all control groups in the test cohort): $b_0$, $b_1$, $b_2$, and $b_2$ are coefficients. This new variable was used to assess whether the combined use of DKK1, DKK3, and AFP was better than the use of either of these biomarkers alone.

All statistical tests were two-sided. A p value of $<0.05$ was considered statistically significant.

## RESULTS

The etiological, demographic, and prognostic features of the study patients are shown in Table 2.

The serum AFP, DKK1, and DKK3 levels were compared between the HCC, cirrhosis, and control groups. The AFP levels showed a significant difference between the HCC, cirrhosis, and control groups ($p<0.001$), with the AFP levels in the HCC group being higher than those in the cirrhosis and control groups. In addition, the AFP levels in the cirrhosis group were significantly higher than those in the control group, but not as high as those in the HCC group (Table 3). The DKK1 levels in the HCC group were higher than those in the cirrhosis and control groups ($p<0.001$); however, the levels were similar between

**Table 1.** Regression equations of the combination of AFP, DKK1, and DKK3

| | Regression equation | Test p | | |
|---|---|---|---|---|
| **Test** | **[Log(p/(1-p))=]** | **AFP** | **DKK1** | **DKK3** |
| HCC vs. Control | 0.556×AFP+0.002×DKK1−6.087 | 0.001 | 0.012 | |
| | 0.002×DKK1+0.000×DKK3−4.885 | | 0.002 | 0.014 |
| | 0.547×AFP+0.000×DKK3−3.981 | 0.002 | | 0.359 |
| | 0.526×AFP+0.002×DKK1+0.000×DKK3−7.284 | 0.003 | 0.012 | 0.294 |
| HCC vs. Cirrhosis | 0.045×AFP+0.001×DKK1−2.923 | 0.007 | 0.007 | |
| Cirrhosis vs. Control | 0.401×AFP+0.000×DKK3−3.195 | 0.006 | 0.008 | |
| Early-Stage HCC vs. Cirrhosis | 0.032×AFP+0.001×DKK1−3.146 | 0.027 | 0.022 | |
| Early-Stage HCC vs. Control | 0.472×AFP+0.002×DKK1−6.248 | 0.005 | 0.037 | |
| | 0.002×DKK1+0.000×DKK3−5.159 | | 0.010 | 0.061 |
| | 0.514×AFP+0.000×DKK3−4.096 | 0.005 | | 0.463 |
| | 0.464×AFP+0.002×DKK1+0.000×DKK3−6.729 | 0.006 | 0.041 | 0.657 |

HCC: hepatocellular carcinoma; AFP: alpha-fetoprotein; DKK: Dickkopf

**Table 2.** Clinical characteristics of the study patients

| | HCC | Cirrhosis | Control |
|---|---|---|---|
| **Sex (M/F)** | 36/4 | 33/21 | 10/29 |
| Age (min–max) | (45–87) 64.2±8.9 | (30–77) 58.4±10.0 | (45–75) 58.4±7.3 |
| **Etiology** | | | |
| HBV/HCV/HBV+HDV | 23/7/1 | 16/3/4 | |
| Alcoholic/Cryptogenic | 3/6 | 4/27 | |
| **Ascites** | | | |
| +/− | 20/20 | 25/29 | |
| **Child–Pugh Stage** | | | |
| A/B/C | 19/15/6 | 25/22/7 | |
| **Lesions (n)** | | | |
| 1/2/3/4/>5 | 24/6/3/1/6 | | |
| **TNM Stage** | | | |
| 1/2/3/4 | 16/13/8/3 | | |
| **Okuda Stage** | | | |
| 1/2/3 | 18/18/4 | | |
| **CLIP Stage** | | | |
| 0/1/2/3/4/5/6 | 11/9/10/7/2/1/0 | | |

M: male; F: female; HCC: hepatocellular carcinoma; HBV: hepatitis B virus; HCV: hepatitis C virus; HDV: hepatitis D virus; TNM: Tumor Node Metastasis; CLIP: Cancer of the Liver Italian Program

the cirrhosis and control groups (Table 3). The DKK3 levels in the cirrhosis and HCC patients were significantly higher than those in the control group (p=0.003), whereas they were similar between HCC and cirrhosis groups (Table 3).

The optimal cut-off levels and areas under the curve for serum AFP, DKK1, and DKK3 in the HCC, cirrhosis, and control groups were calculated using ROC analyses. In addition, the specificities, sensitivities, positive predictive values (PPVs), and negative predictive values (NPVs) were calculated for the serum levels of AFP, DKK1, and DKK3 in each group. The diagnostic accuracies of combinations of markers were calculated with logistic regression analyses, as shown in Table 4.

Alpha-fetoprotein was the best single marker for distinguishing HCC patients from controls (95% CI 0.851–0.980; sensitivity, specificity, PPV, and NPV: 77.5%, 97.4%, 96.9%, and 80.9%, respectively; Table 4, Figure 1). The combination of DKK1 and AFP improved the diagnostic efficiency in discriminating HCC patients from controls (95% CI 0.896–0.996; sensitivity, specificity, PPV, and NPV: 87.5%, 92.3%, 92.1%, and 87.8%, respectively; Table 4, Figure 1). The DKK1 levels were significantly higher in HCC patients, whereas they were similar between the cirrhosis and control groups. DKK3 and the other combinations did not contribute to improved diagnostic efficiency.

Similarly, AFP was the best single marker for distinguishing HCC patients from cirrhosis patients (95% CI 0.737–0.915; sensitivity, specificity, PPV, and NPV: 77.5%, 79.6%, 73.8% and 82.7%, respectively; Table 4, Figure 1), while the combination of DKK1 and AFP improved the sensitivity and PPV in distinguishing HCC from cirrhosis patients (95% CI: 0.785–0.935; sensitivity, specificity, PPV, and NPV: 75%, 85.2%, 78.9%, and 82.1%, respectively; Table 4, Figure 1).

Moreover, AFP was the best single marker for distinguishing early-stage HCC patients from controls (95% CI 0.762–0.980; sensitivity, specificity, PPV, and NPV: 76.2%, 94.9%, 88.9%, and 88.1%, respectively; Table 4, Figure 2) The combination of DKK1 and AFP improved the sensitivity but decreased the specificity in distinguishing HCC patients from controls (95% CI 0.820–1.000; sensitivity, specificity, PPV, and NPV: 85.7%, 87.2%, 78.3%, and 91.9%, respectively; Table 4, Figure 2).

**Table 3.** Discriminative roles of AFP, DKK1, and DKK3

| Marker | Group | Mean±SD | Median | (min–max) | Overall p value | Subgroup p value | |
|---|---|---|---|---|---|---|---|
| AFP (ng/mL) | HCC | 1466.3±1228.4 | 18.4 | (1.7–49192) | | HCC vs. Cirrhosis | <0.001 |
| | Cirrhosis | 7.3±1.9 | 4.0 | (1.3–95.9) | <0.001 | HCC vs. Control | <0.001 |
| | Control | 2.9±0.2 | 2.6 | (0.8–8.2) | | Cirrhosis vs. Control | 0.001 |
| DKK1 (ng/mL) | HCC | 2.1±0.3 | 1.7 | (0.9–13.3) | | HCC vs. Cirrhosis | <0.001 |
| | Cirrhosis | 1.4±0.08 | 1.2 | (0.6–3.7) | <0.001 | HCC vs. Control | 0.001 |
| | Control | 1.3±0.07 | 1.3 | (0.2–2.3) | | Cirrhosis vs. Control | 0.541 |
| DKK3 (ng/mL) | HCC | 66.9±3.7 | 61.9 | (35.4–103.2) | | HCC vs. Cirrhosis | <0.857 |
| | Cirrhosis | 66.0±2.1 | 63.6 | (35.1–113.6) | 0.003 | HCC vs. Control | 0.002 |
| | Control | 55.0±3.7 | 50.1 | (29.3–110.9) | | Cirrhosis vs. Control | 0.004 |

AFP: alpha-fetoprotein; DKK: Dickkopf; HCC: hepatocellular carcinoma; SD: standard deviation

**Table 4.** Discriminative roles of AFP, DKK1, and DKK3

| | Cutoff | AUC (95% CI) | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | LR+ | LR– |
|---|---|---|---|---|---|---|---|---|
| HCC vs. Control | | | | | | | | |
| AFP | 6.435 | 0.916 (0.851–0.980)[b] | 77.5 | 97.4 | 96.9 | 80.9 | 30.225 | 0.231 |
| DKK1 | 1.4 | 0.726 (0.615–0.837)[b] | 72.5 | 61.5 | 65.9 | 68.6 | 1.885 | 0.447 |
| DKK3 | 55.1 | 0.707 (0.590–0.825)[b] | 75.0 | 69.2 | 71.4 | 73.0 | 2.438 | 0.361 |
| AFP+DKK1 | 0.437 | 0.946 (0.896–0.996)[b] | 87.5 | 92.3 | 92.1 | 87.8 | 11.375 | 0.135 |
| DKK1+DKK3 | 0.456 | 0.790 (0.691–0.888)[b] | 77.5 | 66.7 | 70.5 | 74.3 | 2.325 | 0.338 |
| AFP+DKK3 | 0.417 | 0.910 (0.839–0.980)[b] | 82.5 | 92.3 | 91.7 | 83.7 | 10.725 | 0.190 |
| AFP+DKK1+DKK3 | 0.411 | 0.944 (0.891–0.998)[b] | 87.5 | 92.3 | 92.1 | 87.8 | 11.375 | 0.135 |
| HCC vs. Cirrhosis | | | | | | | | |
| AFP | 6.465 | 0.826 (0.737–0.915)[b] | 77.5 | 79.6 | 73.8 | 82.7 | 3.805 | 0.283 |
| DKK1 | 1.4 | 0.741 (0.640–0.842)[b] | 72.5 | 66.7 | 61.7 | 76.6 | 2.175 | 0.413 |
| DKK3 | 55.1 | 0.511 (0.392–0.630) | 75.0 | 35.2 | 46.2 | 65.5 | 1.157 | 0.711 |
| AFP+DKK1 | 0.398 | 0.860 (0.785–0.935)[b] | 75.0 | 85.2 | 78.9 | 82.1 | 5.063 | 0.293 |
| Cirrhosis vs. Control | | | | | | | | |
| AFP | 2.785 | 0.702 (0.596–0.807)[b] | 72.2 | 64.1 | 73.6 | 62.5 | 2.012 | 0.433 |
| DKK1 | 1.3 | 0.463 (0.344–0.581) | 46.3 | 53.8 | 58.1 | 42.0 | 1.003 | 0.997 |
| DKK3 | 58.9 | 0.675 (0.562–0.788)[b] | 63.0 | 74.4 | 77.3 | 59.2 | 2.456 | 0.498 |
| AFP+DKK3 | 0.495 | 0.757 (0.660–0.854)[b] | 75.9 | 64.1 | 74.5 | 65.8 | 2.115 | 0.376 |
| Early-Stage HCC vs. Cirrhosis | | | | | | | | |
| AFP | 6.465 | 0.767 (0.633–0.900)[b] | 71.4 | 79.6 | 57.7 | 87.8 | 3.506 | 0.359 |
| DKK1 | 1.4 | 0.735 (0.599–0.870)[b] | 76.2 | 66.7 | 47.1 | 87.8 | 2.286 | 0.357 |
| DKK3 | 65.7 | 0.509 (0.360–0.657) | 47.6 | 55.6 | 29.4 | 73.2 | 1.071 | 0.943 |
| AFP+DKK1 | 0.207 | 0.825 (0.722–0.929)[b] | 81.0 | 74.1 | 54.8 | 90.9 | 3.122 | 0.257 |
| Early-Stage HCC vs. Control | | | | | | | | |
| AFP | 5.350 | 0.871 (0.762–0.980)[b] | 76.2 | 94.9 | 88.9 | 88.1 | 14.857 | 0.251 |
| DKK1 | 1.6 | 0.725 (0.579–0.870)[b] | 61.9 | 79.5 | 61.9 | 79.5 | 3.018 | 0.479 |
| DKK3 | 55.2 | 0.694 (0.551–0.836)[a] | 76.2 | 69.2 | 57.1 | 84.4 | 2.476 | 0.344 |
| AFP+DKK1 | 0.314 | 0.910 (0.820–1.000)[b] | 85.7 | 87.2 | 78.3 | 91.9 | 6.686 | 0.164 |

**Table 4.** Discriminative roles of AFP, DKK1, and DKK3

| | Cutoff | AUC (95% CI) | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | LR+ | LR– |
|---|---|---|---|---|---|---|---|---|
| DKK1+DKK3 | 0.310 | 0.774 (0.647–0.901)[b] | 76.2 | 66.7 | 55.2 | 83.9 | 2.286 | 0.357 |
| AFP+DKK3 | 0.232 | 0.863 (0.745–0.981)[b] | 81.0 | 84.6 | 73.9 | 89.2 | 5.262 | 0.225 |
| AFP+DKK1+DKK3 | 0.300 | 0.905 (0.810–0.999)[b] | 85.7 | 87.2 | 78.3 | 91.9 | 6.686 | 0.164 |
| Very-Early-Stage HCC vs. Cirrhosis | | | | | | | | |
| AFP | 6.515 | 0.806 (0.584–1.027)[a] | 83.3 | 79.6 | 31.3 | 97.7 | 4.091 | 0.209 |
| DKK1 | 1.4 | 0.606 (0.339–0.874) | 66.7 | 66.7 | 18.2 | 94.7 | 2.000 | 0.500 |
| DKK3 | 89.5 | 0.571 (0.267–0.875) | 50.0 | 88.9 | 33.3 | 94.1 | 4.500 | 0.563 |
| Very-Early-Stage HCC vs. Control | | | | | | | | |
| AFP | 6.485 | 0.904 (0.737–1.071)[b] | 83.3 | 97.4 | 83.3 | 97.4 | 32.500 | 0.171 |
| DKK1 | 1.4 | 0.585 (0.308–0.863) | 66.7 | 61.5 | 21.1 | 92.3 | 1.733 | 0.542 |
| DKK3 | 67 | 0.658 (0.377–0.939) | 66.7 | 76.9 | 30.8 | 93.8 | 2.889 | 0.433 |

[a]p<0.05, [b]p<0.01.

AFP: alpha-fetoprotein; DKK: Dickkopf proteins; AUC: area under the curve; PPV: positive predictive value; NPV: negative predictive value; LR: likelihood ratio; HCC: hepatocellular carcinoma

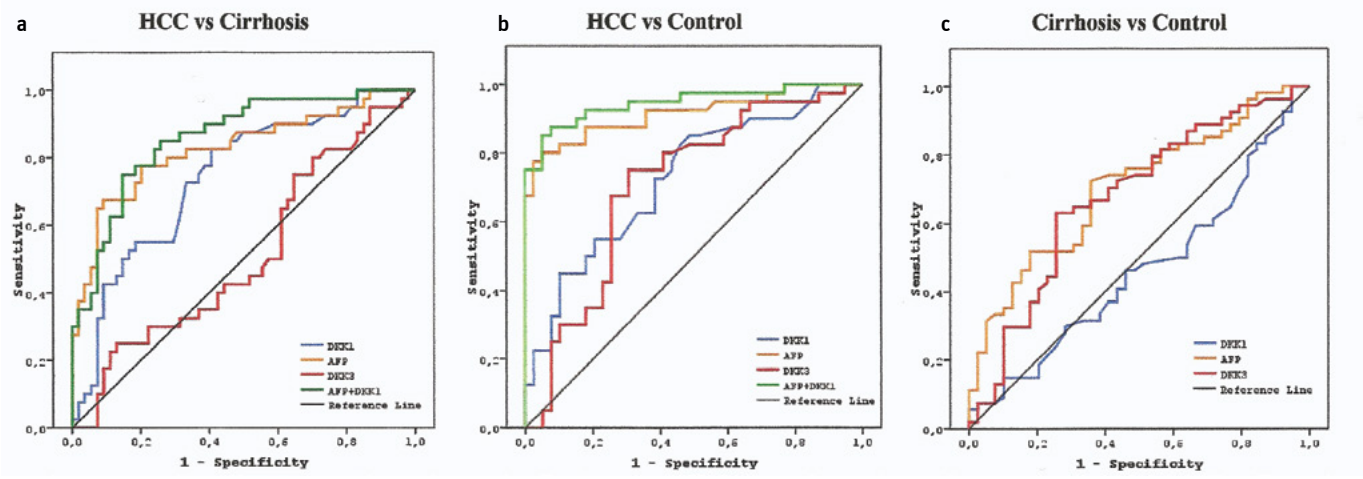


**Figure 1. a-c.** ROC curves for AFP, DKK1, and DKK3 and their combinations in the diagnosis of HCC. HCC vs. Cirrhosis (a), HCC vs. Control (b), Cirrhosis vs. Control (c).
HCC: hepatocellular carcinoma

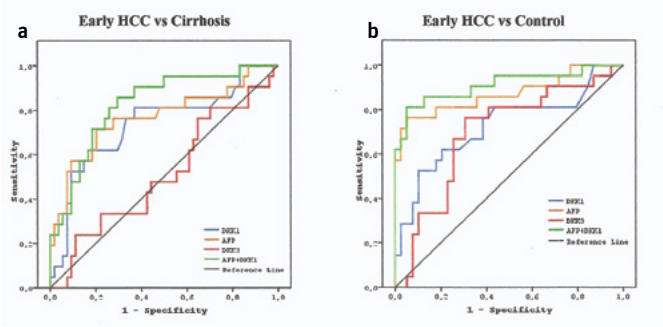


**Figure 2. a, b.** ROC curves for AFP, DKK1, and DKK3 and their combinations in the diagnosis of early-stage HCC (BCLC stage A). Early-Stage HCC vs. Cirrhosis (a), Early-Stage HCC vs. Control (b)
HCC: hepatocellular carcinoma

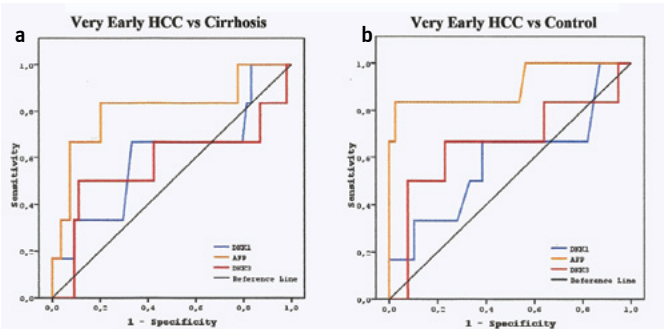


**Figure 3. a, b.** ROC curves for AFP, DKK1, and DKK3 in the diagnosis of very-early-stage HCC (BCLC stage 0) (a. Very-Early-Stage HCC vs. Cirrhosis, b. Very-Early-Stage HCC vs. Control)
HCC: hepatocellular carcinoma

Finally, AFP showed greater diagnostic efficiency than DKK1 and DKK3 in the very early subgroup (Table 4, Figure 3). However, the number of patients in this group was insufficient to calculate the effects of combinations of these markers.

## DISCUSSION

In our study, we showed that AFP levels can distinguish HCC patients from cirrhosis patients, HCC patients from controls, and cirrhosis patients from controls (p<0.001). We also showed

that the DKK1 levels were significantly higher in and could distinguish HCC patients from cirrhosis patients and HCC patients from controls ($p<0.001$). However, there was no statistically significant difference in the DKK1 levels between the cirrhosis and control groups.

Cirrhosis is a known risk factor for HCC. Cirrhosis patients without HCC require close follow-up for the early detection of HCC. While AFP is a current marker for the discrimination of HCC from cirrhosis without HCC, the serum AFP levels are normal in up to 40% of HCC patients, particularly in the early stage of disease (13). Therefore, AFP is not an ideal serum tumor marker for HCC surveillance, and more sensitive markers are required to enable the early detection of HCC.

In this study, we hypothesized that DKK1 could be a promising marker for HCC, particularly when combined with AFP. Indeed, this combination showed a diagnostic advantage for the early diagnosis of HCC compared with AFP alone.

Alpha-fetoprotein showed the greatest sensitivity, specificity, PPV, and NPV among the three markers for comparing HCC patients with cirrhosis patients and controls. Thus, despite its diagnostic limitations, AFP is still the best single marker for the diagnosis of HCC and will continue to play a role in in the diagnostic algorithm of HCC. On the other hand, DKK1 alone was less sensitive and specific and had lower PPV and NPV than AFP alone for comparing HCC patients with cirrhosis patients as well as with controls. Therefore, DKK1 alone is not a valid substitute for AFP as a screening test in HCC surveillance. However, the DKK1 elevation observed in the HCC group indicates that the combination of DKK1 and AFP could improve the diagnostic accuracy of the screening. In fact, the results of the combination were better than those of each marker alone, with the combination of DKK1 and AFP for HCC vs. controls showing greater sensitivity and NPV than AFP alone but lower specificity and PPV. In addition, compared with cirrhosis patients, the combination of DKK1 and AFP showed greater specificity and PPV than AFP alone but lower sensitivity and NPV. In early-stage HCC, the combination of DKK1 and AFP showed greater sensitivity and NPV than AFP alone but lower specificity and PPV for distinguishing between HCC patients and controls; compared with cirrhosis patients, the combination of DKK1 and AFP showed similar results. Hence, the combination of DKK1 and AFP improved the diagnostic accuracy in the different comparisons of groups.

Shen et al. (12) found that compared with chronic hepatitis B and liver cirrhosis patients, the sensitivity and specificity of DKK1 were 69.1% and 84.7%, respectively, while those of AFP were 57.8% and 69.3%, respectively, in HCC patients. Further, it was demonstrated that compared with chronic hepatitis B and liver cirrhosis patients, the sensitivity and specificity of DKK1 were 70.9% and 90.5%, respectively, while those of AFP were 54.4% and 87.9%, respectively, in early-stage HCC patients. Thus, the authors suggested that DKK1 can be used as a diagnostic marker for HCC and that the diagnostic accuracy of DKK1 is superior to that of AFP, particularly for early-stage disease, indicating that DKK1 can improve patient survival by allowing early detection. However, their results were not replicated in this study.

In our study, as mentioned above, DKK1 was not found to be superior to AFP for early-stage HCC (BCLC stage A) and very-early-stage HCC (BCLC stage 0). In addition, we demonstrated that the DKK3 levels can distinguish HCC and cirrhosis patients from controls ($p<0.001$), although we did not detect any significant difference between HCC and cirrhosis patients, thereby limiting its diagnostic role. The ideal marker for HCC should differentiate cirrhosis patients with HCC from those without HCC. Therefore, DKK1 is a better diagnostic test than DKK3 for HCC diagnosis, and although DKK3 methylation plays a role in hepatocellular carcinogenesis (10) and may play role in HCC diagnosis, it does not contribute to early detection.

The patient number is a potential limitation of our study. Further studies with more patients will offer more detailed knowledge about the diagnostic role of these markers.

In conclusion, the combination of DKK1 and AFP showed a better diagnostic yield than AFP alone, and this combination may therefore have clinical relevance. AFP is superior to DKK1 and DKK3 when used individually. DKK3 is an unsuitable marker in clinical use for the diagnosis of HCC.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the ethics committee of Gazi University School of Medicine.

**Informed Consent:** Written informed consent was obtained from patients who participated in this study.

**Peer-review:** Externally peer-reviewed.

**Author Contributions**: Concept - H.E., İ.D.; Design - H.E., İ.D.; Supervision - H.E., İ.D.; Materials - H.E., Ö.G.U., E.K., İ.D.; Data Collection and/or Processing - H.E., Ö.G.U., E.K., B.Ç., Ş.E., İ.D.; Analysis and/or Interpretation - H.E., B.Ç., İ.D.; Literature Review - H.E., İ.D.; Writer - H.E., İ.D.; Critical Review - H.E., İ.D.; Other - H.E., İ.D.

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