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# ROLE OF OSTEOPONTIN AND DIKKOPF RELATED PROTEIN -1(DKK-1) AS DIAGNOSTIC MARKERS OF HCV RELATED HEPATOCELLULAR CARCINOMA

By

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## ABSTRACT

**Background**: Osteopontin (OPN) is an important tumor marker, since it presents as an immobilized extracellular matrix molecule in addition to be present as a secreted form in body fluids involving plasma. Osteopontin levels in the plasma were found to be significantly higher in hepatocellular Carcinoma (HCC) patients than in healthy control individuals and also higher than in patients with chronic liver diseases. Dikkopf related protein-1 (DKK-1) is a diagnostic and prognostic serologic marker for early HCC. The DKK-1 mRNA and protein levels were found to be up regulated in early HCC.

**Study design:** This is a retrospective case control study.

**Objective:** The aim of the present study was to evaluate the role of serum OPN level and Dickkopf-1(DKK1) as potential markers of HCC among HCV infected patients, compared to alpha fetoprotein (AFP). Also, its relationship with clinicopathological features of HCC patients.

**Subjects and Methods**: The study included 90 adult subjects; they were classified into 3 groups. Group1**:** It included 30 patients with HCC. Group 2**:** It included 30 patients with chronic liver disease (CLD) (chronic hepatitis C without HCC), and Group 3**:** It included 30 apparently healthy individuals as a control group. Serum Osteopontin and Dikkopf related protein -1(DKK-1) were measured by Enzyme-linked immunosorbent assay ELISA.

**Results**: There were highly statistical significant differences between the three groups as regard serum Dikkopf related protein -1 and Osteopontin levels ($p < 0.001$). DKK1 and OPN levels were significantly higher in metastasis cases than non-metastatic cases ($p < 0.001$), while AFP level was non-significant $P = 0.424$. Patients with large tumor size have significantly higher OPN levels $p=0.025$, while non-significantlydifferent as regard AFP and DKK1 levels.

**Conclusion:** OPN and DKK1 can be used for diagnosis of HCC and differentiation between HCC and CLD. OPN and DKK1 have higher sensitivity and specificity than AFP and can be used for early diagnosis. Combination between OPN and DKK1 has increased both sensitivity and specificity for detection of of HCC.

**Keywords**: Hepatocellular carcinoma, chronic liver disease, Osteopontin, Dickopf related protein- 1.

## INTRODUCTION

Infection with Hepatitis C Virus (HCV) is the major factor associated with HCC mainly through indirect chronic inflammation, cell death and proliferation. The markers of HCV infection are present in the serum of 80% of patients with HCC **(Lehman and Wilson, 2009).**

Hepatocellular carcinoma is a major health problem in Egypt and its incidence is increasing. The high prevalence of HCV infection makes screening programs and surveillance of those patients a very important tool to early detect cases of small HCCs **(Shaker et al., 2013).**

Osteopontin (OPN) is a glycophospho-protein with cytokine and chemokine properties that was found to be circulating in the biological fluids of healthy individuals, but elevated in cancer patients as well as in individuals with systemic inflammatory response syndrome **(Yang et al., 2014).**

Osteopontin was found to be highly expressed in many malignancies and the expression level of OPN in tumor tissues or in blood of cancer patients has been positively correlated with tumor grade, tumor stage and early recurrence in many cancer types **(Sun et al., 2011).**

DKK-1 is a diagnostic and prognostic serologic marker for early HCC. The DKK-1 mRNA and protein levels were found to be up regulated in early HCC. Serum levels of DKK-1 in patients with early HCC were significantly elevated. DKK-1 had a better sensitivity and accuracy than AFP. More importantly, 73.1% of the patients negative for AFP could be diagnosed with early HCC using DKK-1. A combination of DKK-1 and AFP further improved the diagnostic efficacy **(Yang et al., 2012).**

The aim of the present study was to evaluate the role of serum OPN and Dickkopf-1(DKK1) levels as potential markers of HCC among HCV infected patients, compared to alpha fetoprotein (AFP).

## SUBJECTS AND METHODS

This study was carried out in the Medical Biochemistry and Tropical Medicine Departments between October 2016 and August 2017, AL-Azhar University. A written informed consent was taken from the patients participated in this study. Approval for the study was obtained from the research Ethics Committee, Faculty of Medicine, Al-Azhar University and patients were recruited amongst those attending the Tropical Medicine Department in Al-Hussein University Hospital.

**Subjects:** The study included 90 adult subjects; they were 57 males and 33 females. They were classified into 3 groups:

**Group 1:** It included 30 patients with HCC; 18 males (60%) and 12 females (40%). In 15(50%) of patients the primary HCC lesion was less than 5cm and in the remaining 15 patients (50%) was more than 5cm. 21 (70%) of patients showed HCC metastases and 9 patients (30%) showed no HCC metastases.

**Group 2:** It included 30 patients with CLD (chronic hepatitis C without HCC); 21 males (70%) and 9 females (30%).

**Group 3:** Included 30 apparently healthy individuals (control group); 18 males (60%) and 12 females (40%).

**Exclusion criteria:**

1. Patients having extra hepatic malignancy.
2. Patients having any bony lesions or inflammatory diseases.
3. Patients with any chronic liver disease other than HCV.

All individuals included in this study were subjected to the following:

1. Full history taking focusing on previous hepatic disorders, predisposing factors preceding liver disease, age, sex, alcohol intake and blood transfusion.
2. Thorough clinical examination, with special emphasis on abdominal examination, jaundice, edema and ascites.
3. laboratory investigations: Complete blood count, liver and kidney functions, Hepatitis markers, AFP, Osteopontin and Dikkopf related protein-1.
4. Imaging studies:
   - Abdominal ultrasonography for all patients (liver, spleen, portal vein, ascites).
   - Triphasic computed tomography for HCC group (HCC size, number, site, portal vein thrombosis).

**Sample Preparation:**

5 ml of venous blood was collected in a plain vacutainer tube and allowed to clot,then the serum was separated by centrifugation at 3000 r.p.m for 15 minutes and used for routine laboratory investigations, AFP, Osteopontin and Dickkopf related protein-1 measurement .

Analytical Methods:

**A) Hepatitis markers:**

**For HBsAg:** The analysis of serum HBsAg was done by electrochemiluminescence immunoassay "ECLIA" on the cobas e 411 immunoassay analyzer from Roche diagnostics.

**For anti HCV antibody:** The analysis of serum anti HCV antibody was done by "ECLIA" on the cobas e 411 immunoassay analyzer.

**B) Serum AFP:** The analysis of serum AFP was done "ECLIA" on Cobas e 411 system from cobas.

**C) Serum Osteopontin assay:** Serum osteopontin levels were determined and measured by (ELISA) using ChromateELISA reader Diagnostics (USA) using Sunred ELISA kit(China).

**D) Serum Dickkopf assay:** Serum Dickkopf1 levels were determined and measured by Chromate ELISA reader Diagnostics (USA) using Sunred ELISA kit(China).

**STATISTICAL ANALYSIS:** Data collected throughout history, basic clinical examination, laboratory investigations and outcome measures coded, entered and analyzed using Microsoft Excel software. Data were then imported into Statistical Package for the Social Sciences (SPSS version 20.0) (Statistical Package for the Social Sciences) software for analysis. Quantitative data were represented as number and percentage, mean ± SD, the following tests were used in parametric quantitative independent groups which

were Student t- test in non-parametric normally distributed data while skewed data by Mann Whitney. Differences and association of qualitative variables between two groups by Chi square test ($X^2$).While between multiple groups by one way ANOVA for normally distributed data followed by Tukeys post hoc test, correlation by Pearson's or Spearman's correlation. P value was set at <0.05 for significant results and <0.001 for high significant result.

**ROC curve:**

A receiver operating characteristic (ROC), or simply ROC curve, is a graphical plot which illustrates the performance of a binary classifier system as its discrimination threshold is varied. It was used for determination of cut off values of AFP, OPN and DKK1 for diagnosis of HCC.

## RESULTS

There were significant differences among studied groups as regard clinical characters, i.e. encephalopathy, ascites, edema, portal vein thrombosis and jaundice (Table 1).

**Table (1):** Statistical comparison of clinical findings among studied groups.

| | | | GROUPS | | | Total | P |
|---|---|---|---|---|---|---|---|
| | | | Control | CLD | HCC | | |
| **Encephalopathy** | **No** | **N** | 30 | 18 | 9 | 57 | $P_1$<0.001 $P_2$ <0.001 $P_3$<0.001 |
| | | **%** | 100.0% | 60.0% | 30.0% | 63.3% | |
| | **Yes** | **N** | 0 | 12 | 21 | 33 | |
| | | **%** | 0.0% | 40.0% | 70.0% | 36.7% | |
| **Ascites** | **No** | **N** | 30 | 9 | 6 | 45 | $P_1$<0.001 $P_2$<0.001 $P_3$<0.05 |
| | | **%** | 100.0% | 30.0% | 20.0% | 50.0% | |
| | **Yes** | **N** | 0 | 21 | 24 | 45 | |
| | | **%** | 0.0% | 70.0% | 80.0% | 50.0% | |
| **Edema** | **No** | **N** | 30 | 9 | 3 | 42 | $P_1$<0.001 $P_2$<0.001 $P_3$<0.001 |
| | | **%** | 100.0% | 30.0% | 10.0% | 46.7% | |
| | **Yes** | **N** | 0 | 21 | 27 | 48 | |
| | | **%** | 0.0% | 70.0% | 90.0% | 53.3% | |
| **Portal vein thrombosis** | **No** | **N** | 30 | 27 | 12 | 69 | $P_1$<0.05 $P_2$<0.001 $P_3$ <0.001 |
| | | **%** | 100.0% | 90.0% | 40.0% | 76.7% | |
| | **Yes** | **N** | 0 | 3 | 18 | 21 | |
| | | **%** | 0.0% | 10.0% | 60.0% | 23.3% | |
| **Jaundice** | **No** | **N** | 30 | 12 | 9 | 51 | $P_1$<0.001 $P_2$ <0.001 $P_3$ <0.05 |
| | | **%** | 100.0% | 40.0% | 30.0% | 56.7% | |
| | **Yes** | **N** | 0 | 18 | 21 | 39 | |
| | | **%** | 0.0% | 60.0% | 70.0% | 43.3% | |
| **Total** | | **N** | 30 | 30 | 30 | 90 | |
| | | **%** | 100.0% | 100.0% | 100.0% | 100.0% | |

$P_1$: Control group compared to Chronic liver disease group.
$P_2$: Control group compared to HCC group.
$P_3$: Chronic liver disease group compared to HCC group.

In HCC groups, 6 patients (20%) were classified as stage B, and 24 patients (80%) were classified as stage C. In CLD groups, 9 patients (30%) were classified as stage B, and 20 patients (70%) were classified as stage C (Table 2).

**Table(2):** Child classification between HCC and CLD.

| | | | GROUPS | | Total | P |
|---|---|---|---|---|---|---|
| | | | CLD | HCC | | |
| **Child** | **B** | **N** | 9 | 6 | 15 | 0.37 |
| | | **%** | 30.0% | 20.0% | 25.0% | |
| | **C** | **N** | 21 | 24 | 45 | |
| | | **%** | 70.0% | 80.0% | 75.0% | |
| **Total** | | **N** | 30 | 30 | 60 | |
| | | **%** | 100.0% | 100.0% | 100.0% | |

The mean of DKK1 in control, CLD, HCC was 1.28± 0.383, 1.37± 0.414, and 2.58± 0.510 respectively, and there were statistical significant differences between the three groups ($p_1$< 0.05), ($p_{2,3}$< 0.001 - Table 3).

The mean of OPN in control, CLD, HCC was 31.15± 15.031, 153.60± 93.931, 349.83± 183.912 respectively,there were highly statistical significant differences between the three groups ($p_{1,2,3}$< 0.001).The mean of AFP in control, CLD, HCC was 1.110± 0.224, 34.06± 43.702, 4666.01± 3938.67 respectively, there were highly statistical significant differences between the three groups ($p_{1,2,3}$< 0.001 - Table 3).

**Table (3):** Comparison between DKK1, OPN, and AFP as regard control, CLD and HCC groups.

| | | N | Mean | Std. Deviation | Minimum | Maximum | P |
|---|---|---|---|---|---|---|---|
| **DKK1 (ng/ml)** | **Control** | 30 | 1.28 | 0.383 | .88 | 2.20 | $P_1$<0.05 |
| | **CLD** | 30 | 1.37 | 0.414 | .90 | 2.30 | $P_2$<0.001 |
| | **HCC** | 30 | 2.58 | 0.510 | 1.80 | 3.30 | $P_3$<0.001 |
| **OPN (pg/ml)** | **Control** | 30 | 31.15 | 15.031 | 14.50 | 65.00 | $P_1$<0.001 |
| | **CLD** | 30 | 153.60 | 93.931 | 59.00 | 389.00 | $P_2$<0.001 |
| | **HCC** | 30 | 349.83 | 183.912 | 129.00 | 622.00 | $P_3$<0.001 |
| **AFP (ng/ml)** | **Control** | 30 | 1.110 | 0.224 | .80 | 1.50 | $P_1$<0.001 |
| | **CLD** | 30 | 34.06 | 43.702 | 1.10 | 128.00 | $P_2$<0.001 |
| | **HCC** | 30 | 4666.01 | 3938.67 | 1.80 | 9875.00 | $P_3$<0.001 |

$P_1$: Control group compared to Chronic liver disease group.
$P_2$: Control group compared to HCC group.
$P_3$: Chronic liver disease group compared to HCC group.

There was a statistical significant positive correlation between the levels of OPN with AFP (r= 0.474 & P< 0.001), age (r= 0.196 & P= 0.64), DKK1 (r= 0.764 & P< 0.001), total bilirubin (r= 0.708 & P< 0.001), AST (r= 0.652 & P< ALT (r= 0.637& P< 0.001), urea (r= 0.756& P< 0.001), creatinine (r= 0.630 & P< 0.001), TLC (r= 0.490 & P< 0.001) and PT (r= 0.718& P< 0.001), except for albumin which showed negative significant correlation (r= -0.686 & P< 0.001) and platelets which showed negative significant correlation (r = 0.548 & P< 0.001 - Table 4).

There was a statistical significant positive correlation between the levels of DKK1with AFP (r= 0.533 & P< 0.001), age (r= 0.326 & P= 0.002), total bilirubin (r= 0.675 & P< 0.001), AST (r= 0.609& P< 0.001), ALT (r= 0.620 & P< 0.001), urea (r= 0.540 & P< 0.001), creatinine (r = 0.620 & P< 0.001), TLC (r= 0.564 & P< 0.001) and PT (r= 0.714 & P< 0.001) except for albumin which showed negative significant correlation (r= -0.648 & P< 0.001) and platelets which showed negative significant correlation (r= - 0.366 & P< 0.001 - Table 4).

Lastly, there was a statistical significant positive correlation between the levels of AFP with age (r= 0.223 & P=0.034), total bilirubin (r= 0.554 &P < 0.001), ALT (r= 0.231 & P= 0.029), urea (r= 0.352 & P< 0.001), creatinine (r= 0.216& P= 0.04), TLC (r= 0.453 & P< 0.001), PT(r= 0.615 & P< 0.001) except for albumin which showed negative significant correlation (r= -0.485 & P< 0.001) and platelets which also showed negative significant correlation (r= - 0.312 & P= 0.003 - Table 4).

**Table (4):** Spearman's Correlation between DKK, OPN, AFP and other parameters in HCC group.

| | | DKK1 | OPN | AFP |
|---|---|---|---|---|
| OPN (ng/ml) | R | .764 | 1 | .474 |
| | P | .000 | | .000 |
| AFP (ng/ml) | R | .533 | .474 | 1 |
| | P | .000 | .000 | |
| Age (Years) | R | .326 | .196 | .223 |
| | P | .002 | .064 | .034 |
| TLC ($mm^3/x10^3$) | R | .564 | .490 | .453 |
| | P | .000 | .000 | .000 |
| PLT ($mm^3/x10^3$) | R | -.366- | -.548 | -.312- |
| | P | .000 | .000 | .003 |
| PT (Second) | R | .714 | .718 | .615 |
| | P | .000 | .000 | .000 |
| Creatinine (mg/dl) | R | .620 | .630 | .216 |
| | P | .000 | .000 | .041 |
| Urea (mg/dl) | R | .540 | .756 | .352 |
| | P | .000 | .000 | .001 |
| AST (u/l) | R | .609 | .652 | .183 |
| | P | .000 | .000 | .085 |
| ALT (u/l) | R | .620 | .637 | .231 |
| | P | .000 | .000 | .029 |
| Albumin (g/dl) | R | -.648- | -.686- | -.485- |
| | P | .000 | .000 | .000 |
| Bilirubin (mg/dl) | R | .675 | .708 | .554 |
| | P | .000 | .000 | .000 |

DKK1 and OPN levels were significantly higher in metastasis cases than non metastatic cases (P<0.001), while AFP level was non-significant (P= 0.424 - Table 5).

**Table(5):** Comparison between metastasis cases among HCC group as regard DKK1, OPN and AFP.

| | Metastasis | N | Mean | Std. Deviation | P |
|---|---|---|---|---|---|
| DKK1 (pg/ml) | +VE | 15 | 3.006 | 0.243 | P<0.001 |
| | -VE | 15 | 2.16 | 0.311 | |
| OPN (ng/ml) | +VE | 15 | 506.26 | 127.484 | P<0.001 |
| | -VE | 15 | 193.40 | 37.047 | |
| AFP (ng/ml) | +VE | 15 | 4079.13 | 3422.202 | P= 0.424 |
| | -VE | 15 | 5252.89 | 4436.772 | |

Patients with large tumor size have significantly higher OPN levels P=0.025, while non significantly differrent as regard AFP and DKK1 levels (Table 6).

**Table (6):** Comparison between tumor size among HCC as regard DKK1, OPN and AFP.

| | Size | N | Mean | Std. Deviation | P |
|---|---|---|---|---|---|
| DKK1 (pg/ml) | > 5 | 21 | 2.564 | 0.621 | 0.930 |
| | < 5 | 9 | 2.54 | 0.364 | |
| OPN (ng/ml) | > 5 | 21 | 398.04 | 192.18 | 0.025 |
| | < 5 | 9 | 237.33 | 99.65 | |
| AFP (ng/ml) | > 5 | 21 | 4627.17 | 4043.93 | 0.936 |
| | < 5 | 9 | 4756.64 | 3916.46 | |

The Area Under the Curve for DKK1, OPN and AFP was 0.970, 0.930 and 0.910 and cut off values were >1.75, >164.5 and >7.15 respectively (Table 7).

**Table (7):** Area under the curve and cut off value of DKK1, OPN and AFP.

| Test Result Variable(s) | Area | Cutoff | 95% Confidence Interval | |
|---|---|---|---|---|
| | | | Lower Bound | Upper Bound |
| **DKK1** | 0.970 | >1.75 | .941 | .999 |
| **OPN** | 0.930 | >164.5 | .879 | .981 |
| **AFP** | 0.910 | >7.15 | .840 | .980 |

The sensitivity, specificity, positive predictive value and negative predictive value of DKK1, OPN were higher than AFP. The combination of AFP and DKK1 has increased both sensitivity and specificity of AFP for detection of HCC. The combination of AFP and OPN has increased specificity of AFP for detection of HCC to 95% but decreased sensitivity to 76.7%. The combination of OPN and DKK1has increased both sensitivity and specificity for detection of HCC to 83.3% and 96.6% respectively (Table 8).

**Table (8):** Sensitivity and specificity of DKK, OPN and AFP..

| | **Sensitivity** | **Specificity** | **+VE predictive** | **-VE predictive** | **Accuracy** |
|---|---|---|---|---|---|
| DKK1 | 86.7% | 88.3% | 78.7% | 92.9% | 87.7% |
| OPN | 90.0% | 90.0% | 81.8% | 94.7% | 90.0% |
| AFP | 80.0% | 55.0% | 47.05% | 84.6% | 63.3% |
| AFP & DKK1 | 83.3% | 93.3% | 86.2% | 91.8% | 90.0% |
| AFP & OPN | 76.7% | 95.0% | 88.4% | 89.0% | 88.8% |
| OPN & DKK1 | 83.3% | 96.6% | 87.5% | 90.9% | 93.3% |

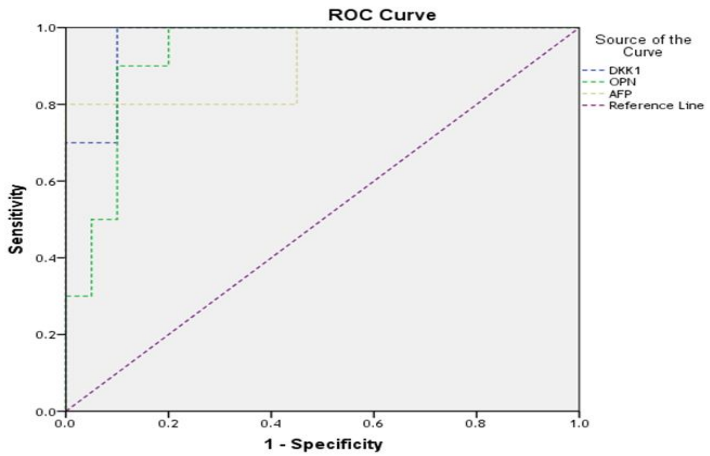


**Figure (1):** ROC Curve for detection of HCC markers cut off values.

## DISCUSSION

Hepatocellular carcinoma is an increasingly prevalent clinical problem worldwide and is the third most common cause of cancer-related death **(Venook et al., 2010).**

Owing to the lack of reliable clinical HCC markers, fewer than 20% of patients are diagnosed at a stage where curative treatment can be performed. In most cases HCC is diagnosed at a late stage, and often arises in a background of chronic liver disease and cirrhosis. Therefore, the prognosis of patients with HCC is generally poor with the 5-year survival rate for this malignancy is depressingly

low, ranging from 4-6% in different countries **(El-Garem et al., 2013).**

The aim of the present study was to evaluate the role of serum OPN level and Dickkopf-1(DKK1) as potential markers of HCC among HCV infected patients, compared to AFP. Also, its relationship with clinicopathological features of HCC patients.

As regards the sex of patients, in our study the male to female ratio in HCC group was 1.5: 1(60% males and 40% females with HCC). The reasons for higher rates of liver cancer in males may be explained by differences in exposure to risk factors. However, sex hormones and other x-linked genetic factors may also be important but there was no significant difference as regard sex. It has been speculated that estrogens and androgens could modulate hepatocarcinogenesis and explain the higher incidence of HCC in men **(El-Zayadi et al., 2005).**

These results were in agreement with those obtained by **Salem et al. (2013)** who reported in HCC patients a male: female ratio was 2: 1, this male predominance was also observed by **Goldman and Ausiello (2004)** who reported a male: female ratio 2:1 up to 4:1. In **Keddeas and Abo-shady (2011)** it was reported that HCC is three times more common in men than women.

Our results showed that there were highly significant differences among groups as regard OPN, Dickkopf1 and AFP markers highest in HCC followed by CLD followed by normal levels in control groups.

The sensitivity of DKK1, OPN and AFP was 86.7%, 90.0% and 80.0% respectively. When AFP was combined with DKK1, sensitivity increased to 83.3%. When AFP was combined with OPN, sensitivity decreased to 76.7%. When OPN was combined with DKK1, sensitivity was 83.3%.

The specificity of DKK1, OPN and AFP was 88.3%, 90.0% and 55.0% respectively. When AFP combined with DKK1, specificity increased to 93.3%. When AFP combined with OPN, specificity increased to 95.0%. When OPN was combined with DKK1, specificity increased to 96.6%.

These results were similar with those of **Salem et al. (2013)** who found that significant elevation of plasma osteopontin levels and AFP levels in HCC patients than HCV patients' levels and lower levels in normal control group.

**Fouad et al. (2015)** also reported that there was a statistically significant increase in the serum OPN levels in the HCC group compared to the benign chronic liver disease groups (HCV without cirrhosis, HCV with cirrhosis, Fatty liver disease), healthy subjects, OPN was superior to AFP in the selective detection, diagnosis of HCC and in predicting liver cirrhosis. **El-Din Bessa et al. (2010)** also found that: plasma levels of OPN and AFP in HCC cirrhotic patients being significantly higher than in cirrhotic patients without HCC and healthy controls.

In our study, the median serum OPN level with small tumor size <5cm was 237.33 ng/mL, and with large tumor size >5cm was 398.04 ng/mL and this was statistically significant.

This was in agreement with **Salem et al. (2013)** who found that tumors < 3 cm, present in 40% of patients, showed median plasma OPN level 140 with a range of (100 - 336 ng/mL), and tumors ≥ 3 cm, present in 60% of patients, showed median plasma OPN level 229 with a range of (131 - 438 ng/mL) (P value: 0.28). However, **Abu El-Makarem et al.**

**(2011)** reported that the median plasma OPN level in tumors< 5cm was 510 ng/mL and in and tumors ≥ 5cm was 1230, and this was statistically significant.

The present study showed that OPN was significantly higher among cases with lymph node metastasis than those with no metastasis. These results were in accordance with **Abu El-Makarem et al. (2011)** reported that the median plasma OPN level in patients with lymph node metastasis (1423ng/mL) was higher than patients with no lymph node metastasis (497ng/ml).

In our study, there was a statistical significant positive correlation between the levels of AFP and OPN, and this was in agreement with that of **Salem et al. (2013)** who found that there was significant positive correlation between OPN and AFP. However, **Sun et al. (2009)** found that the correlation between plasma OPN and serum AFP was insignificant and, therefore, they had stated that plasma OPN levels might be helpful for the diagnosis of HCC in the patients with non-diagnostic AFP level.

In our study, the sensitivity, specificity, PPV and NPV of serum OPN levels in HCC patients were 90, 90, 81.8, and 94.7 respectively at a cut-off value >164.5. AUC for OPN was 0.930 with CI (0.879 – 0.981).

For AFP at a cut-off value >7.15ng/ml, the value of sensitivity, specificity, PPV and NPV of serum AFP levels in HCC patients relative to the CLD group were 80.00 %,45.0 %,47.05 and 84.6 respectively. AUC for AFP was 0.910 with CI (0.840 – 0.980).

Results of our study were in agreement with the study done by **El-Din Bessa et al. (2010)** who reported that, the sensitivity and specificity of OPN for HCC diagnosis were 88.3% and 85.6%, respectively, at a cut-off value of 9.3 ng/mL with OPN having a greater AUC value (0.918) than AFP (0.712).

Many studies reported better diagnostic accuracy of OPN over AFP in HCC diagnosis. **Abohalima and Salem (2014)** found that OPN AUC for HCC diagnosis was 0.991 (95% CI: 0.948 to 1.000) and it differed significantly (p= 0.01) from AFP AUC (0.889, 95% CI: 0.810 to 0.943). At a cut off value of OPN > 178 ng/ml, the test had sensitivity of 98% and specificity of 96% while AFP at a cutoff value of >185 ng/ml had sensitivity and specificity of 86% and 94% respectively in HCC diagnosis.

In contrary to our results, the plasma levels of OPN show low diagnostic accuracy for HCC compared to AFP. However, OPN may have a complementary role in diagnosing HCC in patients with non-diagnostic levels of AFP **(Al-Zoubi et al., 2017).**

There was a highly significant difference between patient and control groups as regard DKK1. This result was in line with that of who reported high expression of DKK1 in HCC **(Yamashita et al., 2008).**

There was no significant difference among patient groups as regard size of lesion. This result was in line with that of **Yu et al. (2009)** who reported that there is no correlation between DKK1-positivity and tumor size. On the other hand **Shen et al. (2012)** stated that there is a correlation between serum DKK1 level and a larger tumor size (≥5 cm). **Gomceli et al. (2012)** reported that DKK1 may have a substantial role is in patients where AFP levels are negative or equivocal such as the case in chronic liver disease.On contrary to our results,**Yang et al. (2004)** stated that dickkopf-1 (Dkk1) is significantly elevated in nodular HCC (multiple lesion) with high metastatic

potential compared to solitary HCC (solitary lesion).

**Fatima et al. (2014)** found that in comparison to serum α-fetoprotein (AFP) level, which remains the gold standard for HCC diagnosis, high serum DKK1 levels have higher diagnostic value for HCC, especially for AFP-negative HCC, and can distinguish HCC from non-malignant chronic liver diseases.

AFP concentrations raised in 11–58% of patients with chronic hepatitis or cirrhosis in the absence of HCC. Therefore, measurement of DKK1 in serum can help to make a differential diagnosis of HCC in patients in these high-risk populations.

**Yu et al. (2009)** found that although elevated levels of AFP remain the gold standard for screening HCC, there are, however, a subgroup of patients who have HCC and normal levels of AFP. When patients were stratified according to AFP levels, DKK1 over expression demonstrated worse prognosis for AFP-normal HCC patients, suggesting that DKK1 may serve as a prognostic marker for this group of patients.

Our study showed that there was high levels of OPN and DKK1 in metastatic cases compared to non-metastatic ones with statistical significant difference $P<0.001$.

## CONCLUSION

1. OPN and DKK1can be used for diagnosis of HCC and differentiation between HCC and CLD.
2. OPN and DKK1have higher sensitivity and specificity than AFP and can be used for early diagnosis of HCC.
3. OPN and DKK1can be used for differentiation between metastatic and non-metastatic HCC.

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# دور الأستيوبنتين والبروتين المرتبط بالديكوف -١كدلالات أورام فى تشخيص سرطان الكبد الناتج عن الإصابة بالإلتهاب الكبدى الفيروسى سى

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**خلفية البحث:** يعتبر الأسوتيوبونتين من دلالات الأورام الهامة، حيث أنه من المصفوفات المتجمدة خارج الخلية ، هذا بالإضافة إلى وجوده بشكل مفرط فى سوائل الجسم وتشمل بلازما الدم. وقد وجد أن مستويات الأستيوبونتين فى بلازما الدم أعلى فى مرضى سرطان الكبد حيث أنها تكون أعلى من الأصحاء وأعلى أيضاً من مرضى الالتهاب الكبدى المزمن. ويعتبر البروتين المرتبط بالديكوف١ علامة مصلية للتشخيص والإنذار المبكر لسرطان الكبد. وقد وجد أن الرسول الرنا للبروتين المرتبط بالديكوف١ يرتفع أيضاً فى الإصابة المبكرة بسرطانات الكبد.

**الهدف من البحث**: هو التحقق من إمكانية استخدام مستوى الأستيوبونتين والديكوف١ بوصفها علامة بيولوجية وأهميتهما الإكلينيكية كدلالة قوية لتشخيص مرضي سرطان الكبد مقارنة ب ألفا فيتوبروتين. وكذا علاقتهما بالأعراض الإكلينيكية والباثولوجية لمرضى سرطان الكبد.

**مواد وطرق البحث:** اشتملت هذه الدراسة على تسعين شخصا تم تقسيمهم الى ثلاث مجموعات:

**المجموعة الأولى:** ثلاثون مريضا و تشمل المرضى المصابين بسرطان الكبد الناتج عن الإصابة بالالتهاب الكبدى الفيروسى سي.

**المجموعة الثانية:** وتشمل ثلاثون مريضا مصابون بالالتهاب الكبدى المزمن.

**المجموعة الثالثة**: وتشمل ثلاثون شخصا من الأشخاص الأصحاء ليكونوا المجموعة الضابطة .

وقد تم قياس الديكوف١والفا فيتو بروتين والأستيوبونتين بطريقة الاليزا.

**النتائج:** كان هناك فروق ذات دلالة إحصائية في مستويات الديكوف١ والأستيوبونتين بين

المجموعات التى تم دراستها. كما وجد أن مستويات الديكوف١ والأستيوبونتين تكون مرتفعة فى مرضى سرطان الكبد المصاحب بثانويات عن غير المصاحب بثانويات، بينما لم يختلف مستوى ألفا فيتوبروتين بين هاتين المجموعتين. وقد وجد أن المرضى الذين يعانون من حجم كبير من أورام الكبد يرتفع مستوى الأستيوبونتين لهم، بينما لم يختلف مستويات الديكوف١ والألفا فيتوبروتين.

**توصيات البحث:** يمكن استخدام الأوستيوبونتين و الديكوف١ لتشخيص أورام الكبد السرطانية، كما يمكن استخدامهما للتفريق بين أورام الكبد السرطانية والالتهاب الكبدى المزمن. كما أن الأستيوبونتين و الديكوف١ يتميزان بان لهما حساسية ونوعية اعلى من الالفا فيتوبروتين ويمكن استخدامهما للتشخيص المبكر لأورام الكبد السرطانية. كما أن الدمج بين الاستيوبونتين والديكوف ١ ينتج عنه زيادة النوعية والحساسية للتشخيص المبكر لأورام الكبد السرطانية.