ejpmr, 2016,3(9), 20-27.

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

COMPARATIVE STUDY BETWEEN DKK-1 AND AFP FOR DIAGNOSING OF HEPATOCELLULAR CARCINOMA AMONG EGYPTIAN PATIENTS.

Faten Zahran Mohamed,[1] LamiaaAbdullateef Ali Barakat,[2] Noha Hassan Radwan[3] and Mohsen Mohamed Mohamed Khedr*[4]

[1]Department of Chemistry, Faculty of Science, Zagazig University, Zagazig, Egypt.
[2]Department of Chemistry, Faculty of Science, Port Said University, Port said, Egypt.
[3]National Center Institute, Faculty of Medicine, Cairo University, Cairo, Egypt.
[4]Department of Chemistry, Faculty of Science, Port Said University, Port said, Egypt.

*** Corresponding Author: Mohsen Mohamed Mohamed Khedr**

Department of Chemistry, Faculty of Science, Port Said University, Port said, Egypt.

Article Received on 22/06/2016 **Article Revised on 13/07/2016** **Article Accepted on 02/08/2016**

ABSTRACT

Background: Hepatocellular carcinoma is a major health problem. It was ranked 2nd most common cancer site among males and 7th among females. AFP is the main routinely parameter for HCC diagnosis, it can also be elevated in liver cirrhosis. It represents a liver cell- specific, not a tumor-specific marker, for these reasons, our suggestion is to use AFP as a supplementary marker for diagnosis of HCC. So identification of sensitive biomarkers to improve early diagnosis of HCC is needed. Our aim is to evaluate serum Dickkopf-1 (DKK1) as biomarkers for early diagnosis of HCC. **Methods:** The study included 40 HCCs patients (stage I (9) cases, stage II (31) cases), 30 liver cirrhosis patients, and 20 healthy subjects were enrolled. Serum DKK1 using ELISA kit was measured in all included subjects. **Results:** Serum DKK1 was significantly elevated in HCC group compared to cirrhotic and healthy control groups. Receiver operating characteristic curve showed the best cutoff values for DKK1, and AFP were (1 ng/ml and 400 ng/ml) respectively. Area under the curve of DKK1 was significantly larger than that of AFP (0.990 vs. 0.763). The sensitivity of DKK1 and AFP was (98% vs. 28%) respectively. **Conclusion:** Serum DKK1 might be potential diagnostic markers for HCC.

KEY WORDS: HCC patients, DKK1, AFP.

INTRODUCTION

Hepatocellular carcinoma is one of the most common cancers worldwide, it accounts for 85-90% of all primary liver cancers, with a median survival of less than one year[1], it's very poor prognosis makes it the third leading cause of cancer-related death, responsible for approximately 600,000 deaths annually[2].

A study was performed in 2012 showed that it occurred in 782,000 people and resulted in 746,000 deaths[3]. The major risk factors for hepatocellular carcinoma (HCC) are chronic hepatitis B virus (HBV) infection, chronic hepatitis C virus (HCV) infection, prolonged dietary aflatoxin exposure, alcoholic cirrhosis, and cirrhosis due to other causes[4].

In Egypt, HCC can cause about 4.7% of chronic liver disease patients[5], the incidence rate was doubled in the past 10 years[6], the epidemic of HCC in Egypt is related to hepatitis C viral infection (HCV), where Egypt has the highest prevalence of HCV in the world with about 13.8% of the population infected and seven million persons with chronic HCV liver disease[7].

In most cases, HCC is diagnosed at a late stage; therefore the prognosis of patients with HCC is generally poor and has a less than 5% survival rate[8], the poor prognosis has been led to its insidious onset and late presentation at diagnosis. It has been widely established that the early detection of HCC enables more treatment options and better survival[9].

The most widely tools which is used for observation of HCC is Liver ultrasound in combination with Alpha-fetoprotein (AFP), however, AFP alone (without liver ultrasound) is not recommended due to its low sensitivity and specificity for detecting HCC, where there are other causes such as Chronic hepatitis or cirrhosis can raise AFP in 20% and 40% of patients, respectively, and tend to fluctuate in parallel with underlying inflammatory activity[10], at the same time HCC can produce a range of AFP values from normal to >100,000 ng/ml[11], where it was found that normal AFP levels are present in about 30% of patients at time of diagnosis and usually remain low, even with advanced HCC[12]. Furthermore, only one third of patients with HCC have AFP levels higher than 100 ng/ml[13], also it was found that at a serum cutoff level of 20 ng/ml, the sensitivity was 65 %, and the

specificity was 78.1%[14], but at a serum cutoff level of >400–500 ng/ml which is considered diagnostic for HCC, the specificity of AFP is close to 100% but at cost to the sensitivity which falls below 45%[15]. Thus, the identification of new noninvasive biomarkers with better sensitivity and specificity could be helpful in the early diagnosis of HCC.

A new biomarker for diagnosing (HCC) is Dickkopf-1 (DKK1) which has been discovered in 2012. It has great potential especially in early-stage disease. It is a secreted inhibitor of the Wnt/β-catenin pathway; also it is a negative regulator of bone formation and plays a role in cell proliferation and survival control. DKK1 is frequently over expressed in many human tumors where it can promote or suppress tumor growth, depending on the tumor type[16]. The identification of serum DKK1, allowed the detection of early stages of HCC[17], and the identification of HCC in patients negative for AFP, also in patients positive for AFP, DKK1 can distinguish between HCC and chronic hepatitis B or liver cirrhosis[18].

MATERIAL AND METHODS

The study included 90 subjects who are ranged in age from 45- 65 years. Patients with other malignancies, a family history of malignancy and those with age younger than 45 or older than 65 Years were excluded from the study. Consent from the patients or care provider was collected before inclusion in the study. The study subjects were classified into three groups.

Group 1: included 40 HCC patients who further classified pathologically to stage I (9) patients, stage II (31) patients, they were 32 males and 8 females.

Group 2: included 30 patients with liver cirrhosis (LC); they were 24 males and 6 females.

Group 3: included 20 volunteering apparently normal healthy individuals as controls; they were 15 males and 5 females. The age of all groups was ranged from 45 to 65 Years.

Collection and handling of samples.

Six ml venous blood was withdrawn from each individual;1.8 ml of blood was collected in sodium citrate tube for determination of prothrombin time, then 1.2 ml of blood was collected in EDTA tube for determination of (HB, RBC,s WBC,s and Platelets count) and other three ml of blood was collected in gel vacutainer tube, then left for 10 minutes in water bath at 37°C until clot, then centrifuged at 2000 rpm for 10 minutes for separation of serum which was transferred to another tube and kept frozen at (-20°C) to detect DKK1, AFP, HCV antibody and HBV antigen.

All patients and controls were subjected to full medical history taking thorough clinical examination for patients only, routine laboratory investigations including (Hb, RBC,s, WBC,s, Platelets) using Coulter Sysmex, kidney function (Urea and Creatinine), liver functions (ALT, AST, ALP, Albumin, Total protein, Bilirubin) were quantified by using commercially available kits on auto-analyzer Beckman CX9 autoanalyser (Beckman Inst, Kraemer BLW, Brea, USA), Prothrombin time & concentration and INR were done using Siemens, tumor markers (Serum alpha-fetoprotein were done using Axsym instrumental that based on the microparticle enzyme immunoassay (MEIA) technology, serum DKK1 was done using ELISA kit (enzyme-linked immunosorbent assay), and abdominal ultrasound. Only those in HCC group underwent further imaging in the form of abdominal triphasic spiral CT or magnetic resonance imaging as a part of HCC diagnosis.

Serum DKK-1 was measured in all enrolled subjects using ELISA kit supplied by Wuhan EIAab science (Wuhan EIAab science CO., Ltd. Wuhan, China by ELISA technique, Catalog No: E0741h for DKK1 and Catalog No: E0631h for MDK). ELISA (R&D Systems) was performed according to manufacturer's recommendations. Briefly, the assay is based on a double -antibody sandwich ELISA technique for the quantitative assay of human DKK1 in samples.

Statistical Analysis

All statistical analyses of the data were done according to (20) by statistical package for the social sciences (SPSS), version 15.0 on Microsoft Windows XP (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as mean±SD, whereas categorical variables were expressed as numbers (percentages). A value of $P< 0.05$ was considered statistically significant.

RESULTS

Patient's Characteristics of the HCC group was reported in table 1, where Males were 32 cases (80%) and female were 8 cases (20%). Nine patients (22.5%) were classified as stage I, 31 patients (77.5%) as stage II. Seven patients (17.5%) showed hepatomegaly, while 11 patients (27.5%) showed splenomegaly. Ascites was detected in 39 cases (97.5%); oedema was detected in two cases (5%), and portal vein thrombosis in 8 cases (20%). According to the number of masses, 26 cases (65%) had one mass, 4 cases (10%) had two masses and 10 cases (22.5%) had multiple masses. Regarding to the size of mass, 9 cases (22.5%) had masses with 1-3 cm and 31 cases (77.5 %) had masses more than 3 cm. Seven cases (17.5%) were positive for hepatitis B marker only, 18 cases (45%) were positive for hepatitis C marker only and 2 cases only (5%) were positive for both markers B and C, and 13(32.5) cases were negative for both markers.

Table 1: Patient's Characteristics of the HCC group. N (%)

Sex	Male	32 (80%)
	Female	8 (20%)
Focal lesions	Single	26 (65%)
	Two	4 (10%)
	Multiple	10 (25%)
Size of mass	1 - 3 cm	9 (22.5%)
	More than 3 cm	31 (77.5%)
Stage	Stage I	9 (22.5%)
	Stage II	31 (77.5%)
Hepatomegaly	Absent	33 (82.5%)
	Present	7 (17.5%)
Splenomegaly	Absent	29 (72.5%)
	Present	11 (27.5%)
Ascites	Absent	1 (2.5%)
	1	9 (22.5%)
	2	12 (30%)
	3	18 (45%)
Oedema	Absent	38 (95%)
	Present	2 (5%)
Portal vein thrombosis	Absent	32 (80%)
	Present	8 (20%)
Hepatitis markers	Hepatitis B	9 (22.5%)
	Hepatitis C	20 (50%)

Comparison of DKK1, AFP and biochemical parameters between the different studied groups.

With respect to the values of DKK1 and AFP in the normal healthy group which are (0.169 ± 0.023 and 2.5 ± 0.59) respectively, it was clear that these values were statistically higher in the liver cirrhotic group when compared with the healthy control by about (117%, 246%) respectively and were statistically higher in HCC group when compared with the healthy control by about (973.9%, 111089%).

ALT, AST, ALP and total Bilirubin showed the highest results in the HCC group followed by the cirrhotic and then the normal control group. Albumin, Platelets, prothrombin concentration and Hb showed the highest results in the normal control group followed by the HCC group and then cirrhotic group, while prothombin time, INR, Urea and creatinine showed the highest results in cirrhotic group followed by HCC group and then normal control group. The comparison of the different studied groups were statistically significant for ALT (P=0.003), AST (P=0.006), ALP (P=0.011), prothrombin time (P=0.001), prothrombin concentration (P=0.000), INR (p=0.000), Albumin (P=0.000), Total bilirubin (P=0.007), Platelets (P=0.001) and Hb (p=0.000).

Table 2: Comparison of DKK1, AFP and biochemical parameters between the different studied groups.

	Normal Control group	Liver Cirrhosis group	HCC group	P- value
DKK1 (ng/ml)	0.169 ± 0.023	0.367 ±0.052	1.815 ± 0.625	**<0.001***
AFP (ng/ml)	2.5 ± 0.59	8.65 ± 7.75	2779.74 ± 7928.8	**<0.053***
ALT (U/L)	22.0 ± 5.16	52.02 ± 30.92	58.20 ± 36.69	**0.003***
AST (U/L)	19.45 ± 5.09	73.62 ± 86.62	88.44 ± 65.12	**0.006***
ALP (U/L)	110.35 ± 18.57	141.6 ± 39.03	173.40 ± 107.68	**0.011***
Albumin (g/dl)	4.08 ± 0.18	2.93 ± 0.72	3.08 ± 0.46	**0.000***
Total Bilirubin (mg/dl)	0.63 ± 0.11	1.47 ± 1.14	1.79 ± 1.43	**0.007***
Urea (mg/dl)	26.3 ± 4.8	45.63 ± 49.67	31.85 ± 13.08	**0.059**
Creatinine (mg/dl)	0.89 ± 0.10	1.09 ± 0.47	0.93 ± 0.31	**0.189**
Prothrombin time (Sec)	12.53 ± 0.51	14.35 ± 1.99	13.91 ± 1.78	**0.001***
Prothrombin Con (%)	96.08 ± 5.47	79.18 ± 16.82	83.66 ± 13.28	**0.000***
INR (Ratio)	1.03 ± 0.06	1.22 ± 0.20	1.18 ± 0.17	**0.000***
Hb (g/l)	13.8 ± 1.0	11.75 ± 2.14	12.14 ± 1.91	**0.000***
Platelets (x 10^3 / µL)	234.25 ± 42.40	108.83 ± 44.29	171.37 ± 99.245	**0.000***
WBCs (x 10^3 / µL)	6.13 ± 1.06	5.36 ± 2.13	6.73 ± 3.73	**0.139**
RBCs (x 10^6 / µL)	4.61 ± 0.36	4.16 ± 0.83	4.12 ± 0.62	**0.021**

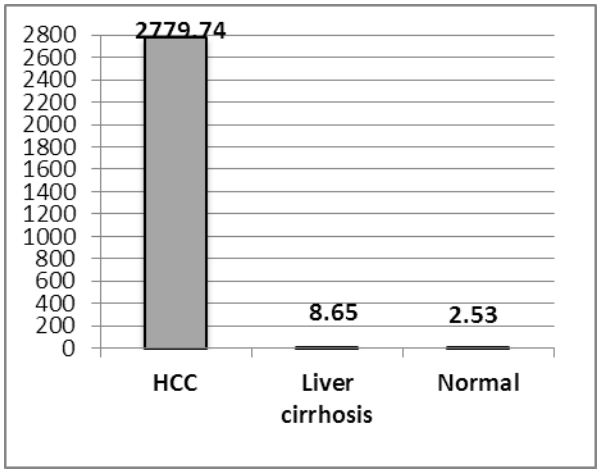

Figure (1): Mean level of AFP in all studied groups.

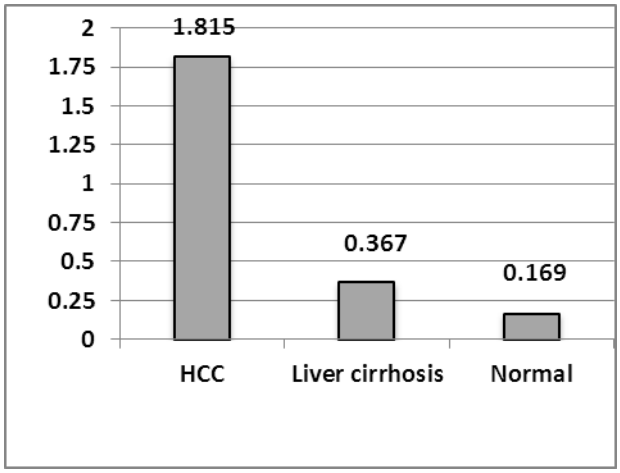

Figure (2): Mean level of DKK1 in all studied groups.

The best chosen cut offs for DKK1 and AFP were (1ng/ml and 400 ng/ml) respectively. DKK1showed the highest AUC (0.99 CI (0.982-1.006) then AFP (0.763 CI (0.665-0.866). DKK1showed the highest sensitivity (98%), then AFP (28%). As regards the specificity, all variables have the same specificity (100 %). also all variables have the same Positive predictive value (100%), regarding to the negative predictive value DKK1showed the highest value (97%) followed by AFP (51%), also DKK1showed the highest diagnostic accuracy (99%), then AFP (59%) **Table (3).**

Table 3: The diagnostic performance of DKK1 and AFP.

Parameter	Cutoff value ng/ml	AUC (95% CI)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	P
DKK1	1	0.99 (0.982 - 1.006)	98	100	100	97	99	<0. 001*
AFP	400	0.763 (0.665 - 0.866)	28	100	100	51	59	<0. 001*

Receiver operating characteristic (ROC) curve analysis was applied to evaluate the diagnostic utility of DKK1 and AFP in HCC patients versus the cirrhotic patients and the healthy control group

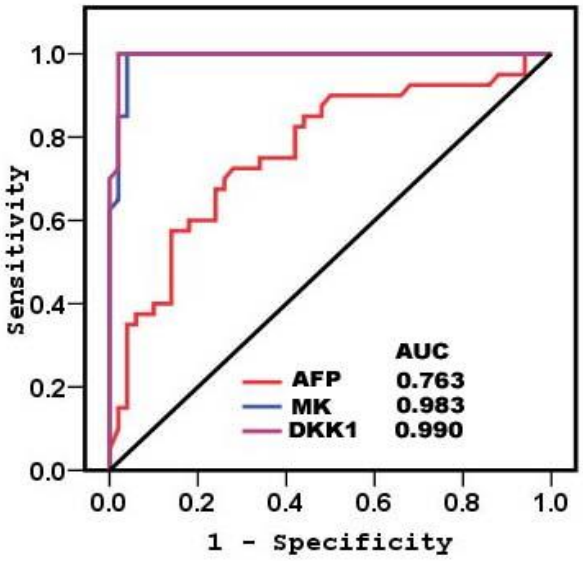

Figure 4: Shows the ROC of DKK1 and AFP on one curve.

DISCUSSION

HCC is usually asymptomatic and is diagnosed at a late stage in most cases; the prognosis of patients with HCC is generally poor and has a less than 5% survival rate[8]. Therefore, there is an urgent need for find sensitive biomarkers for early diagnosis and monitoring recurrence of HCC[20]. Current diagnosis relies on clinical finding, liver imaging and measurement of serum AFP.

In Egypt, HCV infection was estimated to account for (40-50%) of HCC cases, while HBV has declining influence of infection representing about (25%)[5]. this is in accordance with the results of our study where (50%; n=20/40) of HCC cases were positive for HCV and only (22.5%; n=9/40) were positive for HBV as shown in **table (1)**.

AFP is the most widely used tumor marker for diagnosis of HCC; however the mean value of serum AFP in healthy control group was found to be (2.5±0.6 ng/ml), while the mean value of cirrhotic group was (8.65± 7.75 ng/ml) and (2779.74 ± 7928.8 ng/ml) for HCC group as shown in (**table 2)**,(**fig.1)**. It was noted that there was a marked increase in serum level of AFP in HCC group compared with other groups (P<0.05), also there is a significant increase in cirrhotic group compared with healthy control group.

The increased serum level of AFP is thought to be due to hepatic regeneration following hepatocytes destruction in viral hepatitis[28] also it may be due to the response to hepatocytes injury or possibly due to increased hepatocytes turnover[29].

Our results are in agreement with (47) who reported that there was a marked increase in serum level of AFP in HCC group with the mean value (2913±1424.7), then cirrhotic group with the mean value (6.48±1.32), also our results are in accordance with[32] who reported that there was a statistically highly significant elevation of the mean serum level of AFP in HCC group compared with control, cirrhotic and HCV group, and are in line with[28] who reported that, AFP has been detected in HCC, liver cirrhosis and Hepatitis B or C virus infections.

Using cut-off of (20 ng/ml) about (42.5%; n=17/40) of HCC patients were AFP negative, which is in accordance with[47] who reported that more than half (56.98%; n=49/86) of HCC patients were negative for AFP.

However, it was reported that, patients with chronic liver disease, particularly those associated with a high degree of hepatocyte regeneration, can express AFP in the absence of cancer and it is also elevated in hepatocarcinogenesis[31].

In summary, AFP is the main routinely parameter for HCC diagnosis, also can be detected in liver cirrhosis and liver diseases. It represents a liver cell- specific, not a tumor-specific marker, for the previous reasons, our suggestion is to use AFP as a supplementary marker to help diagnosis of HCC.

As regard the obtained results of serum DKK1, it showed that the mean value of DKK1 in healthy control group was found to be (0.169 ± 0.023 ng/ml), which are in line with those of[33] who reported a value of (0.01- 1.91) ng/ml in healthy control group. The mean value of DKK1 was found to be 0.367±0.052 in cirrhotic group and 1.815 ± 0.625 in HCC group as shown in **(table 2) (fig. 2),** which means that there is a significant increase in DKK1 levels in HCC group compared with other groups (P<0.001),(cirrhotic and healthy control group).

It was demonstrated that overexpression of DKK1 not only enhances the tumor formation efficiency and tumor growth but also promotes the cell invasion and metastasis in vitro and in vivo; DKK1 did not influence HCC cell proliferation and colony formation, while dramatically promotes HCC cell migration and invasion.

Our results are in agreement with[18] who reported that serum levels of DKK1 were significantly higher in Patients with HCC than in all controls, also our results are in accordance with those of[35],[36] who reported that elevated expression of DKK1 was found in both tissue and serum samples from patients with HCC.

On the other hand, our result differs from the study that was performed by[40], who reported that the mean level of DKK1 in HCC group was (1.76 ± 0.15) ng/ml, in the cirrhotic group was (2.031 ± 0.163), and in Healthy control group was (3.11± 0.2), suggesting that DKK1 has limited value as biomarker for HCC diagnosis.

The ROC curve shows that Dkk1 had the best diagnostic performance with greater AUC, sensitivity, specificity positive predictive value, Negative predictive value and more Accuracy than AFP for diagnosing of HCC as shown in (**table 3**) which is in accordance with[36], who demonstrated that serum DKK1 had larger AUC than that of AFP (0.877 vs 0.793, p<0.05) and had a better sensitivity (76.0% vs 50.0%, p<0.05), specificity (93.8% vs 89.3%, p<0.05), accuracy (85.2% vs 70.4%, p<0.05), positive predictive value (91.9%vs 81.3 %, p<0.05) and Negative predictive value (80.8% vs 65.8% , p<0.05). Our results also are in line with[38], who demonstrated that DKK1 had the best diagnostic performance with the greatest AUC (0.902 vs 0.792), sensitivity (79.78% vs 71.91%), specificity (93.07% vs 75.25%), positive predictive value (91.03% vs 71.91%) and Negative predictive value(83.93 % vs 75.25%).

The Area under the curve of the combination of (AFP and Dkk1) (0.995, 95 % CI 0.995–1.002) as shown in **(table 4) (fig. 5)**, was larger than that of AFP or DKK1 alone **(fig. 4)**, which is in accordance with[37], who demonstrated that the AUC for the combination of AFP and DKK1 (0.931, 95 % CI 0.901–0.954) was larger than

that of AFP or DKK1 alone, also our results are in accordance with[18], who demonstrated that a combination of AFP and DKK1could further improve the diagnostic accuracy of HCC. And in agreement with[36] who reported that the combined testing of DKK1 and AFP was the best way to diagnose early HCC, especially for those patients who were negative for AFP.

On the other hand our results differs from the study that was performed by[38], who demonstrated that, by comparing DKK1 alone with the combined DKK plus AFP tests, DKK1 plus AFP tests had a higher pooled sensitivity, and slightly lower pooled specificity, which demonstrated that both DKK1 alone and combined DKK1 plus AFP tests were similar in terms of accuracy for diagnosing HCC.

As regard to the obtained results of liver enzymes, the mean value of the activity of ALT and AST in control group was found to be (22.0 ± 5.16 U/L) and (19.45 ± 5.09 U/L) respectively as shown in **table (2)**, which is in accordance with the results of (21) who reported a mean value of (23 ± 5.1U/L) and (15.5 ± 4.6 U/L) for ALT and AST respectively in control group.

The mean value of the activity of ALT and AST showed the highest results in HCC group with mean of ALT (58.20 ± 36.69U/L), AST (88.44 ± 65.12) ($p<0.003$) and ($P<0.006$) respectively followed by cirrhotic group then normal control group as shown in **table (2)** which is statistically significant, also the increase in serum levels of AST is greater than that of ALT. This variation in elevation is due to the fact of presence of two iso-forms of AST-cytosolic and mitochondrial. In severe necrosis the mitochondrial fraction is also released into the circulation along with the cytosomal fraction[22].

Our results are in accordance with[23] who reported that an increase in liver enzymes is caused by inflammation or damage to liver cells, where injured hepatocytes leak higher than normal amounts of enzymes in to the blood, causing an elevation of liver enzymes. HCC, liver cirrhosis, liver fibrosis, and HCV and HBV infection can cause elevation of liver enzymes. So a diagnosis of elevated liver enzymes is itself does not confirm any sort of liver damage.

The mean value of albumin in control group was found to be (4.08 ± 0.181g/dl), which is in agreement with the results of[24] who demonstrated a normal range of Albumin is (3.4-5.4g/dl).

The mean value of albumin was found to be decreased in cirrhotic group (2.93 ± 0.72 g/dL) when compared to HCC group (3.08 ± 0.46g/dL) and normal control group (4.08 ± 0.18 g/dL), as shown in **table (2)** which in accordance with[25] who reported that Serum albumin levels were decreased to a greater extent in cirrhotic group (2.09 ± 0.202 g/dL) when compared to normal group (4.12 ± 0.379 g/dL), the low serum albumin indicates that the synthetic function of liver is affected in liver disease, also the low serum albumin has been suggested to indicate the severity of liver cell damage and inability of injured hepatocytes to produce sufficient albumin[27].

CONCLUSION

This study showed that the serum DKK1 may serve as good diagnostic biomarkers for the diagnosis of HCC Particularly at an early stage, also DKK1 alone or in combination with AFP significantly improve the diagnostic accuracy of early HCC with sensitivity, specificity and accuracy higher than that of AFP alone.

Recommendations

- Screening and follow up of cirrhotic patients with DKK1 due to its higher sensitivity and specificity in detecting early cases of HCC.
- DKK1 is recommended to be used as a routine tumor marker for early detection and follow up of HCC.
- The combination of DKK1 and AFP could further improve the diagnostic Accuracy of HCC.
- Further studies with larger population are needed to justify its implementation in clinical practice.

Conflicts of interest

The authors declare that there was no conflict of interests regarding the publication of this article.

REFERENCES

1. El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology 2007; 132(7): 2557-76.
2. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin. 2005; 55(2): 74-108.
3. World Cancer Report 2014 and World Health Organization. 2014; pp. Chapter 1.1
4. Taniguchi K, Roberts LR, Aderca IN, Dong X, Qian C, Murphy LM, Nagorney D M, Burgart LJ, Roche PC, Smith DI, Ross JA and Liu W. Mutational spectrum of *betacatenin*, *AXIN1* and *AXIN2* in hepatocellular carcinomas and hepatoblastomas. Oncogene. 2002; 21: 4863–4871.
5. El-Zayadi AR, Badran HM, Barakat EM, Attia Mel-D, Shawky S, Mohamed MK, Selim O, Saeid A. Hepatocellular carcinoma in Egypt, a single center study over a decade. World J Gastroenterol 2005; 11: 5193-5198.
6. Anwar WA, Khaled HM, Amra HA, El-nezami H, Loffredo CA. Changing pattern of hepatocellular carcinoma (HCC) and its risk factors in Egypt, possibilities for prevention. Mutat res. 2008; 659: 176–184.
7. Perz JF, Alter MJ. The coming wave of HCV-related liver disease, dilemmas and challenges. J Hepatol. 2006; 44: 441–443.

8. Bertino G, Ardiri A, Malaguarnera M, Malaguarnera G, Bertino N, Calvagno GS. Hepatocellualar carcinoma serum markers. Semin Oncol. 2012; 39(4): 410-433.
9. Yuen MF, Cheng CC, Lauder IJ, Lam SK, Ooi CG, Lai CL. Early detection of hepatocellular carcinoma increases the chance of treatment, Hong Kong experience. Hepatology. 2000; 31: 330-5.
10. Johnson PJ. The role of serum alpha-fetoprotein estimation in the diagnosis and management of hepatocellular carcinoma. Clin Liver Dis. 2001; 5(1): 145-59.
11. Koteish A, Thuluvath PJ. Screening for hepatocellular carcinoma. J Vase Interv Radiol. 2002; 13(9 Pt 2): S185–S190.
12. Colombo M. screening for cancer in viral hepatitis. Clin Liver Dis. 2001 Feb; 5(1): 109-22.
13. Torzilli G, Minagawa M, Takayama T, Inoue K, Hui AM, Kubota K, Ohtomo K and Makuuchi M. Accurate preoperative evaluation of liver mass lesions without fine needle biopsy. Hepatology 1999; 30: 889–893.
14. Paul SB, Gulati MS, Sreenivas V, Madan K, Gupta AK, Mukhopadhyay S, Acharya SK. Evaluating patients with cirrhosis for hepatocellular carcinoma: Value of clinical symptomatology, imaging and alpha-fetoprotein. Oncology. 2007; 72 Suppl 1: 117-23.
15. Gupta S, Bent S, Kohlwes J. Test characteristics of alpha-fetoprotein for detecting hepatocellular carcinoma in patients with hepatitis C. A systematic review and critical analysis. Ann Intern Med 2003 Jul 1; 139(1): 46-50.
16. Menezes ME, Devine DJ, Shevde LA, Samant RS. Dickkopf1: a tumor suppressor or metastasis promoter? Int J Cancer. 2012; 130: 1477–1483.
17. Tung EK, Ng IO. Significance of serum DKK1 as a diagnostic biomarker in hepatocellular carcinoma. Future Oncol. 2012; 8(12): 1525-1528.
18. Shen Q, Fan J, Yang XR, Tan Y, Zhao W, Xu Y, Wang N, Niu Y, Wu Z, Zhou J, Qiu SJ, Shi YH, Yu B, Tang N, Chu W, Wang M, Wu J, Zhang Z, Yang S, Gu J, Wang H, Qin W. Serum DKK1 as a protein biomarker for the diagnosis of hepatocellular carcinoma, a large-scale, multicentre study. Lancet Oncol. 2012; 13(8): 817-26.
19. Levesque, R. SPSS programming and Data Management, a Guide for SPSS and SAS users, Fourth Edition. *SPSS Inc.* Chicago, IL. 2007; 60606-6412.
20. Yuen MF, Lai CL. Serological markers of liver cancer. Best Pract Res Clin Gastroenterol. 2005; 19(1):91-9.
21. Elameen MK, Abdrabo AA. Comparative Study of Liver Enzymes Activities in Smokers and Diabetic Sudanese Patients. Asian Journal of Biomedical and Pharmaceutical Sciences. 2013; 03 (27); 39-41.
22. Paul L. Wolf. Biochemical Diagnosis of liver disease. Indian J Clin Biochem. 1999 Jan; 14(1): 59–90.
23. Russo MR, JACOBSON IM. How to use statins in patients with chronic liver disease. Cleve and Clinic. J. of Medicine. 2004; 71(1): 58-62.
24. Berk PD, Korenblat KM. Approach to the patient with jaundice or abnormal liver test results. In: Goldman L, Ausiello D, eds. Cecil Medicine. 23rd ed. Philadelphia, Pa: Saunders Elsevier. 2007 chap 150.
25. Sreedevi NN, Chakrapani A. Study of Adenosine Deaminase Levels Along With Alanine and Aspartate Aminotransferase, Total Proteins and A/G Ratio in Cirrhosis. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS). 2014; 13(4): 04-10.
26. Fontana RJ, Lok AS. Noninvasive monitoring of patients with chronic hepatitis C. Hepatology. 2002; 36: 57-64.
27. Yao DF, Dong ZZ, Yao M. specific molecular markers in hepatocellular carcinoma. Hepatobiliary pancreat Dis Int. 2007; 6(3): 241-247.
28. Hu KQ, Kyulo NL, Lim N, Elhazin B, Hillebrand DJ, Bock T. Clinical significance of elevated alpha-fetoprotein (AFP) in patients with chronic hepatitis C, but not hepatocellular carcinoma. Am J Gastroenterol. 2004; 99(5):860-5.
29. Di Bisceglie AM, Sterling RK, Chung RT, Everhart JE, Dienstag JL, Bonkovsky HL, Wright EC, Everson GT, Lindsay KL, Lok AS, Lee WM, Morgan TR, Ghany MG, Gretch DR. Serum alpha-fetoprotein levels in patients with advanced hepatitis C: results from the HALT-C Trial. J Hepatol. 2005; 43(3): 434-41.
30. Grizzi F, Franceschini B, Hamrick C, Frezza EE, Cobos E, Internati CM. Usefulness of cancer-testis antigens as biomarkers for the diagnosis and treatment of hepatocellular carcinoma. J Transl Med. 2007; 5: 3.
31. Gameel M, El Assaly N, Madani H, El-Ashry N, Mostafa I, Abd El Raof E, El-Rasky M. Evaluation of Tumor Markers panel in Detection of HCC in HCV Egyptian patients and its Correlation with AFP. Research Journal of Medicine and Medical Science. 2009; 4(2): 402-410.
32. Seung Up Kim, Jeon Han Park, Hyon-Suk Kim, Jae Myun Lee, Hyun Gyu Lee, Hyemi Kim, Sung Hoon Choi, Shinhwa Baek, Beom Kyung Kim, Jun Yong Park, Do Young Kim, Sang Hoon Ahn, Jong Doo Lee, and Kwang-Hyub Han. Serum Dickkopf-1 as a Biomarker for the Diagnosis of Hepatocellular Carcinoma. Yonsei Med J. 2015; 56(5): 1296-1306.
33. Liang Chen, Ming Li, Qian Li, Chao-jie Wang, Song-qiang Xie. DKK1 promotes hepatocellular carcinoma cell migration and invasion through β-catenin/MMP7 signaling pathway. Mol Cancer. 2013; 12: 157.
34. Tao YM, Liu Z, Liu HL. Dickkopf-1 (DKK1) promotes invasion and metastasis of hepatocellular carcinoma. Dig Liver Dis. 2013; 12: 251–257.
35. Tung EK, Mak CK, Fatima S, Lo RC, Zhao H, Zhang C, Dai H, Poon RT, Yuen MF, Lai CL, Li

JJ, Luk JM, Ng IO. Clinicopathological and prognostic significance of serum and tissue Dickkopf-1 levels in human hepatocellular carcinoma. Liver Int. 2011; 12: 1494–1504.
36. Yang H, Chen GD, Fang F, Liu Z, Lau SH, Zhang JF, Lau WY, Yang LY. Dickkopf-1: as a diagnostic and prognostic serum marker for early hepatocellular carcinoma. Int J Biol Markers. 2013; 28(3): 286-297.
37. Ge T, Shen Q, Wang N, Zhang Y, Ge Z, Chu W, Lv X, Zhao F, Zhao W, Fan J, Qin W. Diagnostic values of alpha-fetoprotein, dickkopf-1, and osteopontin for hepatocellular carcinoma. Medoncol. 2015; 32(3): 59.
38. Jie zhang, Ying zhao, Qin Yang. Sensitivity and specificity of Dickkopf-1 protein in serum for diagnosing hepatocellular carcinoma: a meta-analysis. Int j Biol markers. 2014; 29(4): e403-e410.
39. Ikematsu S, Yano A, Aridome K, Kikuchi M, Kumai H, Nagano H, Okamoto K, Oda M, Sakuma S, Aikou T, Muramatsu H, Kadomatsu K, Muramatsu T. Serum midkine levels are increased in patients with various types of carcinomas. Br J Cancer. 2000; 83: 701–706.
40. Vongsuvanh R, van der Poorten D, Iseli T, Strasser SI, McCaughan GW, George J. Midkine Increases Diagnostic Yield in AFP Negativeand NASH-Related Hepatocellular Carcinoma. PLoSONE. 2016; 11(5): e0155800.