

Diagnostic and Predictive Value of Some Tumor Markers in the Diagnosis and Follow Up of Patients with Hepatocellular Carcinoma

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ABSTRACT

Early detection of hepatocellular carcinoma (HCC) is essential for successful treatment. Although the role of alpha fetoprotein (AFP) in the diagnosis of advanced HCC is well recognized, at least one third of small HCCs and 15–20% of advanced HCC will be missed unless another diagnostic tool is used. Thus, new serologic markers with sufficient sensitivity and specificity are required. In the present study, we aimed at evaluating the diagnostic and prognostic role of AFP, protein induced by vitamin K absence or antagonist (PIVKA-II), vascular endothelial growth factor (VEGF), sialic acid, alpha-L-fucosidase (AFU) and transforming growth factor-beta 1 (TGF- β 1) in the diagnosis and follow up of Egyptian patients with hepatocellular carcinoma in an attempt to find a tumor marker with a reasonable sensitivity and specificity for diagnosis of HCC and monitoring patients after therapy. The study was conducted on 4 selected groups of patients and a control group. Group I included 10 patients with liver cirrhosis. Group II consisted of 10 patients with benign hepatic focal lesions. Group III included 10 HCC patients without distant metastasis and group IV included 10 HCC patients presenting with metastasis. Ten apparently healthy age and sex matched subjects were also included and served as control group. AFP, sialic acid, alpha-L-fucosidase (AFU), VEGF, PIVKA-II and TGF- β 1 were determined in the blood of five studied groups. The sensitivity and specificity of the tumor markers were calculated and compared. Significant differences in the median blood levels of AFP, PIVKA-II, VEGF, sialic acid, TGF- β 1 and alpha-L-fucosidase activity (AFU) were found on comparing the HCC groups (with and without metastasis) with the other groups. The median blood levels of PIVKA-II, α -fetoprotein level, sialic acid and AFU activity were lower in the HCC group without metastasis compared to that with metastasis ($p < 0.001$). On the other hand, the median serum VEGF level was higher in the HCC group without metastasis compared to that of the HCC group with distant metastasis ($p < 0.001$). Serum TGF- β 1 level did not vary significantly between both groups (with and without metastasis) ($p > 0.05$). There were significant lower median blood levels of all parameters in HCC patients without metastasis after ablation therapy compared to pretreatment levels. Combined determination of serological markers could be used as a highly valuable tool for screening and diagnosis of HCC as combination of these markers improved their sensitivity and specificity. They could also be used as prognostic markers decreasing the need for more invasive procedures.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant neoplasms. It is associated closely with cirrhosis and its prognosis is very poor because the diagnosis is generally late, when HCC presents with clinical symptoms, the tumor is usually advanced and the patient has few therapeutic options¹. Therefore, early detection is important in the management of that type of cancer. Advanced imaging modalities have been used for screening HCC² and several tumor markers have been recently investigated in HCC for assessing diagnosis, and monitoring patients with diagnosed disease³. Although the role of alpha fetoprotein (AFP) in the diagnosis of advanced HCC is well recognized, at least one third of small HCC and 15 - 20% of advanced HCC will be missed unless another diagnostic tool is used. Furthermore, AFP may be elevated in non-malignant liver diseases⁴.

Protein induced by vitamin K absence or antagonist (PIVKA-II) is a tumor marker complementary to AFP in the diagnosis of HCC⁵. Tumor progression is angiogenesis dependent and vascular endothelial growth factor (VEGF) is a key growth factor in that process. It has been suggested that serum VEGF level may be useful as a biological marker of tumor invasiveness and a prognostic factor in HCC⁶. Transforming growth factor- β 1 (TGF- β 1) is a potent growth inhibitor of normal hepatocytes and hepatoma cells that induces apoptosis in these cells. TGF- β 1 is highly expressed in many malignant tumors including HCC⁷⁻⁹.

Sialic acid is a member of a family of N- and O- acetyl derivatives of neuraminic acid and widely distributed in mammals. It usually occurs as a terminal component at the non-reducing end of carbohydrate chains of glycoproteins and glycolipids and is thought to be involved in receptor function, cell recognition and immunological reactions, it also accounts for 70-80 % of the total surface negativity in a variety of cell types. Sialic acid is increased in the serum of tumor-bearing humans and animals, and thus is a probable indicator of malignant tumor growth¹⁰. Alpha-L-fucosidase (AFU) (E.C. 3.2.1.51) is a lysosomal enzyme present in all mammalian cells, involved in the catabolism of the fucose-containing glycoconjugates.¹¹ In accordance with the study of *Deugnier et al. (1984)*¹¹, the serum level of Alpha-L-fucosidase activity in patients with HCC was increased. This may be related to increased enzyme release by tumor cells¹². AFU is an enzyme that catalyzes the chemical reaction of two substrates of this enzyme, α -L-fucoside and H₂O, whereas its two products are L-fucose and alcohol¹¹.

In the current study, we aimed at evaluating the diagnostic and prognostic role of AFP, PIVKA-II, VEGF, sialic acid, AFU and TGF- β 1 in the diagnosis and follow up of Egyptian patients with hepatocellular carcinoma in an attempt to find a tumor marker with a reasonable sensitivity and specificity for early diagnosis of HCC and monitoring patients after therapy.

PATIENTS & METHODS

The study was conducted on four selected groups of patients according to the clinical, laboratory, ultrasonographic and histopathological evaluation. The patients were selected from the Tropical Medicine Department, Tanta University Hospital, Tanta University. The included groups were as following:

Group I: 10 patients with liver cirrhosis (Child-Pugh class A).

Group II: 10 patients with benign hepatic focal lesions (HFL) as 3 cases with haemangioma, 3 cases with amoebic liver abscess, 2 cases with pyogenic liver abscess and 2 cases with hydatid cyst).

Group III: 10 HCC patients without metastasis.

Group IV: 10 HCC patients with distant metastasis.

Control Group: formed of 10 apparently healthy, age and sex matched subjects without any evidence of liver disease. All patients and control groups gave an informed consent to participate in the study.

Exclusion criteria:

1. Patients of Child-Pugh class B or C.
2. For HCC groups, patients with the following criteria were excluded:
 - a. Multiple focal lesions.
 - b. Portal vein thrombosis.

Blood Samples

Plasma samples: Six mls venous blood samples were taken from every patient and control subject, then immediately 1.8 mL of the blood sample was added to 0.2 sodium citrate in precalibrated tubes. The tubes containing the samples were centrifuged for 30 minutes at 3000 Xg

at 4°C and aliquots of the resulting plasma were transferred to polypropylene microtubes and stored at -80°C. Samples were thawed at room temperature at the time of assay measurement. Plasma samples were needed for PIVKA-II and TGF-β1 assay. It is very important to prevent platelets degranulation during the plasma collection for TGF-β1 assay because platelets constitute one of the main sources of TGF-β1. So, the plasma was separated from the cells within 1 hour, centrifuged at 3000Xg for 30 minutes at 4°C to eliminate platelets, then the platelet-depleted plasma was collected.

Serum samples: The remaining peripheral venous blood sample were taken and transferred to disposable tubes. The tubes were promptly centrifuged at 4°C, and aliquots of serum were immediately stored at -80°C until measurement. Serum samples were needed for other parameters assay.

All patients were subjected to:

- Clinical assessment by history taking and clinical examination.
- Laboratory evaluation including: Liver function tests (bilirubin, transaminases, alkaline phosphatase, prothrombin time and activity, serum total proteins and serum albumin).
- Serological examination for hydatid antibodies and amoebiasis.
- Hepatitis markers:
 - a) Hepatitis C virus (HCV): Anti-HCV antibodies by third generation ELISA (Murex Diagnostic, England DAI 5LR) and PCR for HCV RNA.

- b) Hepatitis B virus (HBV): HBsAg, anti-HBcAb by ELISA (Sorin Biomedica) and PCR for HBV.
- Imaging techniques including:
 - a) abdominal ultrasonography with Doppler examination
 - b) Triphasic CT (abdomen and pelvis).
 - c) Patients with HCC were subjected to bone scan and MRI of brain, chest and abdomen to detect distant metastasis.
 - Liver biopsy and histopathological evaluation for HCC patient groups.
 - Cytological examination of fluid aspirated from infectious cysts.
 - Assay of tumor markers: AFU activity was measured by modification of the method of *Zielke et al.*¹². 10 µl of serum were added to 50 µl of the substrate mixture, *p*-nitrophenyl- α -L-fucopyranoside dissolved in phosphate buffer (pH 5) and incubated for 1 hour at 37°C. The reaction was stopped by adding 3.5 ml of stop buffer (pH 10.5) (15 mM sodium carbonate, 15 mM glycine and 7.5 mM sodium chloride). Blanks were prepared in the same way, but the incubation step was omitted. Absorbance of *p*-nitrophenol was read at 400nm. The change in the absorbance of the liberated *p*-nitrophenol was directly proportional to the activity of α -L-fucosidase enzyme and expressed as nmol/mL/h¹². Sialic acid was measured by method of *Hess et al.*¹³ which depends on precipitation of serum proteins with trichloroacetic acid. The color resulting from condensation of N-acetylneuraminic acid with sulfuric-acetic acid reagent is read in the

spectrophotometer at 530 nm. α -fetoprotein (AFP) was measured by quantitative sandwich ELISA using kits supplied by Biosource (Biosource International, California, USA)¹⁴, PIVKA-II was assayed by quantitative sandwich ELISA using kits supplied by Diagnostic Stago (Diagnostic Stago, France)¹⁵, VEGF by competitive enzyme immunoassay using kits supplied by Cytimmune (Cytimmune Science INC. Maryland, USA)¹⁶, TGF- β 1 by quantitative sandwich ELISA using kits supplied by Biosource (Biosource International, Inc., California, USA)¹⁷.

Hepatocellular carcinoma patients of Group III were treated using percutaneous ethanol injection (PEI) or percutaneous radiofrequency ablation (RF) according to the optimal protocol of percutaneous ablation therapy. PEI ablation was selected for treatment of HCC patients when a tumor satisfied the following criteria: (1) existing in a subcapsular location, (2) existing in a location adjacent to a major vessel and another organ (heart, gall bladder, stomach and bowel) and (3) demonstrating poor differentiation. Four patients (40%) were treated by PEI with tumors \leq 3 cm in diameter. RF ablation was selected for the cases with low risks of tumor seeding and hemorrhage. Six patients (60%) were treated by RF ablation. No local tumor residues were found in all patients as assessed by contrast enhanced CT six months after the initial successful percutaneous ablation therapy (no vascularities could be recognized within the tumor by contrast enhanced CT). All the studied tumor markers were assayed again in patients group

III six months after the initial ablation successful treatment.

Statistical analysis: Continuous variables are expressed as mean \pm SD for normally distributed data and by median for non parametric data. Student t-test (paired, unpaired), paired t-test was used in comparing levels of the different parameters in patients group III before and after ablation therapy. Mann Whitney U test and Kruskal Wallis tests were used for analysis. Multiple receiver operating characteristic curves (ROC curves) were drawn to assess the validity of the tumor markers.

RESULTS

The age and sex of the different studied groups are shown in table1.

The 5 groups were comparable regarding age and sex. Ninety percent (18/20) of HCC patients (with and without metastasis) were positive for anti-HCV and only 10% (2/20) were HBsAg positive. Clinical presentations of the different studied groups are shown in table2. Table 3 shows the results of liver function tests in the studied groups. The mean values of total bilirubin and aspartate transaminase were significantly higher in liver cirrhosis and the two HCC groups (III, IV) than the benign HFL and the control groups ($p < 0.05$). The mean value of alkaline phosphatase was significantly higher in the two HCC groups (with and without metastasis) than the other groups ($p < 0.05$).

Table 1: Age and sex of the different studied groups.

	No	Age		Sex	
		Range (year)	Mean \pm SD (year)	Female	Male
Control Group	10	25 - 58	42.1 ^a \pm 8.6	4(40%)	6(60%)
Group I	10	28 - 51	43.2 ^a \pm 7.7	2(20%)	8 (80%)
Group II	10	26 - 52	46.35 ^a \pm 9.6	1(10%)	9 (90%)
Group III	10	32 - 60	48.1 ^a \pm 8.13	3(30%)	7(70%)
Group IV	10	43 - 60	48.6 ^a \pm 4.6	2(20%)	8(80%)

Groups bearing different initials are significantly different from each other.

Table 2: Clinical presentation of the different studied groups

	Group I n (%)	Group II n (%)	Group III n (%)	Group IV n (%)
Rt. Hypochondrial pain	2(20%)	3 (30%)	9 (90%)	9 (90%)
Jaundice	5(50%)	1 (10 %)	7 (70%)	8 (80%)
Hepatomegaly	0 (0%)	0(0 %)	2 (20%)	5 (50%)
Splenomegaly	6(60%)	0 (0%)	6 (60%)	7 (70%)
Lower limb oedema	6(60%)	0 (0%)	7 (70%)	8 (80%)
Upper GIT bleeding	0(0%)	0 (0%)	1 (10%)	2 (20%)
Ascitis	6(60%)	0 (0%)	8 (80%)	9 (90%)
Bleeding tendency	3(30%)	0 (0%)	8 (80%)	9 (90%)
Coma	3(30%)	0 (0%)	6 (60%)	7 (70%)
Fever	1(10%)	3 (30%)	1 (10%)	1 (10%)

Table 3: Liver function tests of the studied groups. Groups bearing different initials are significantly different from each other.

	Control Group mean±SD	Group I mean±SD	Group II mean±SD	Group III mean±SD	Group IV mean±SD
Total bilirubin (mg/dl)	0.73±0.3 ^b	2.03±0.53 ^a	0.66±0.25 ^b	2.51±2.95 ^a	5.83±11.94 ^a
Aspartate transaminase (U/L)	19±4.1 ^b	89.1±93.6 ^a	25.7±13.1 ^b	105.4±50.9 ^a	109.8±56.3 ^a
Alanine transaminase (U/L)	25.5±8.5 ^c	36.8±14.3 ^a	25.1±8.9 ^a	63.5±23.9 ^a	72.8±54 ^a
Alkaline phosphatase (U/L)	104.9±38.9 ^c	172±183.6 ^a	165.8±32.4 ^a	372.9±305 ^b	456.2±276 ^b
Total proteins (g/dl)	7.3±0.5 ^{bc}	6.6±0.6 ^a	7.5±0.6 ^b	7.1±0.7 ^{ac}	6.7±1.1 ^b
Albumin (g/dl)	3.7±1.4 ^b	3.1±0.6 ^a	3.8±0.5 ^b	2.9±0.5 ^{adc}	2.7±0.2 ^c
Prothrombin concentration %	91.5±5.8% ^d	78.7±5.8% ^a	82.5±7.9% ^a	68±16.2% ^b	66.5±18.4% ^b

On ultrasonographic examination, 80% of group II, III and IV had a single focal lesion. Only 20% of the same groups had double lesions. The median blood levels of AFP, PIVKA-II, VEGF, sialic acid, AFU and TGF- β 1 in the five studied groups are presented in table 4. A statistically significant differences in the median blood levels of AFP, PIVKA-II, TGF-

β 1, sialic acid, VEGF and AFU activity were observed on comparing the HCC groups (with and without metastasis) with the control, the benign HFL and the cirrhotic groups ($p < 0.001$) (table 4).

On comparing the HCC group without metastasis (group III) with that with distant metastasis (group IV), it was found that the median

blood levels of AFP, PIVKA-II, sialic acid and AFU activity were significantly higher in the latter group (group IV) than that in the former (group III) ($p < 0.001$). On the other hand, the median serum VEGF level was significantly higher in the HCC group without metastasis (2580 pg/ml) when compared to the group with distant metastasis (1840 pg/ml) ($p < 0.001$). Plasma TGF- β 1 level did not vary significantly between both groups ($p > 0.05$) (Table 4).

Comparison of the median blood levels of AFP, PIVKA-II, TGF- β 1, VEGF, sialic acid and AFU activity in HCC patients without metastasis (group III) before and six months after percutaneous ablation therapy revealed a statistically highly significant lowering of all parameters after ablation therapy (Table 5). Multiple receiver operating characteristic curve (ROC) revealed that the area under the curve (AUC) for AFP, PIVKA-II, TGF- β 1, VEGF, AFU and sialic acid were 0.880, 0.900, 0.910, 0.890, 0.960 and 0.760 respectively (Fig. 1).

For AFP, at a cut-off level of 28 ng/mL, the sensitivity was 80%, the specificity 97%, positive predictive value (PPV) 92.3%, negative predictive value (NPV) 86.6% and the

overall accuracy was 90%. For PIVKA-II, at a cut-off level of 12.5 mAU/mL, the sensitivity was 80%, specificity 97%, PPV 96%, NPV 86.9% and accuracy was 90%. Concerning VEGF, at a cut-off level of 780 pg/mL, the sensitivity was 96.7%, specificity 85%, PPV 82.4%, NPV 97.1% and the accuracy was 90%. For sialic acid, at a cut-off level of 90 mg/dL sensitivity was 80%, specificity 78 %, PPV 72.7 %, NPV 77.7% and accuracy was 75%. At a cut-off level of 443 nmol/ml/h for AFU, the sensitivity was 80%, the specificity was 90%, PPV 81.8%, NPV 88.8% and accuracy was 85%. Lastly for TGF- β 1, at a cut-off level of 32.4 ng/mL sensitivity was 76.7%, specificity 97%, PPV 95.8%, NPV 84.7% and accuracy was 88.5% (Table 6).

A negative correlation was detected between plasma PIVKA-II and serum VEGF ($r = -0.594$, $p = 0.012$) in the HCC groups (III and IV), but no statistically significant correlations were found between serum AFP and the other markers (AFU, sialic acid, VEGF, PIVKA-II and TGF- β 1) ($p = 0.428$, 0.692, 0.322, 0.091 and 0.106 respectively) (Table 7).

Table 4: The median values of AFP, PIVKA-II, TGF- β 1, VEGF, sialic acid and AFU in the five studied groups. Groups bearing different initials are significantly different from each other.

	Control Group n=10	Group I n=10	Group II n=10	Group III n=10	Group IV n=10	p-value
AFP (ng/mL)						
Median	2.5 ^a	6 ^a	2.5 ^a	673 ^b	2217 ^c	<0.001
Range	1.3-3.4	4.1-9.8	1.4-3.9	290-916	51.8-3015	
PIVKA-II (mAU/mL)						
Median	1.1 ^a	1.1 ^a	1.5 ^a	16.2 ^b	33.6 ^c	<0.001
Range	0.4-2.6	0.8-2.1	1.1-2.5	12.7-21.0	25.6-53.2	
TGF β 1 (ng/mL)						
Median	20.2 ^a	20.7 ^a	20.8 ^a	42.5 ^b	37.6 ^b	<0.001
Range	17.1-22.4	11.8-26.7	15.6-26.3	37.5-50.7	27.9-44.5	
VEGF (pg/mL)						
Median	160 ^a	180 ^a	160 ^a	2580 ^b	1840 ^c	<0.001
Range	70-210	80-960	100-520	2140-2970	1300-2310	
Sialic Acid (mg/dL)						
Median	61 ^a	63 ^a	87 ^a	115 ^b	208 ^c	<0.001
Range	59.1-109.5	59.9-118.1	73.1-147.2	84.3-219.8	175.1-265.3	
AFU (nmol/mL/h)						
Median	321 ^a	502 ^a	675 ^a	897 ^b	1655 ^c	<0.001
Range	210-448	466 - 758	625 - 831	403-1434	1545- 2058	

Table 5: The median value of AFP, VEGF, TGF- β 1, PIVKA-II, sialic acid and AFU in the HCC group without metastasis (group III) before and after treatment

	Before treatment	After treatment	p-value
	n=10	n=10	
AFP (ng/mL)			
Median	673	31	<0.001
Range	290-916	13.2-40.3	
PIVKA-II (mAU/mL)			
Median	16.2	4.1	<0.05
Range	12.7-21.0	2-5.7	
TGF-β1 (ng/mL)			
Median	42.5	17.5	<0.05
Range	37.5-50.7	14.5-19.8	
VEGF (pg/mL)			
Median	2580	440	<0.001
Range	2140-2970	250-540	
Sialic Acid (mg/dL)			
Median	115	29.1	<0.001
Range	84.3-219.8	27.2-38.2	
AFU (nmol/ml/h)			
Median	897	409	<0.001
Range	403-1434	398- 567	

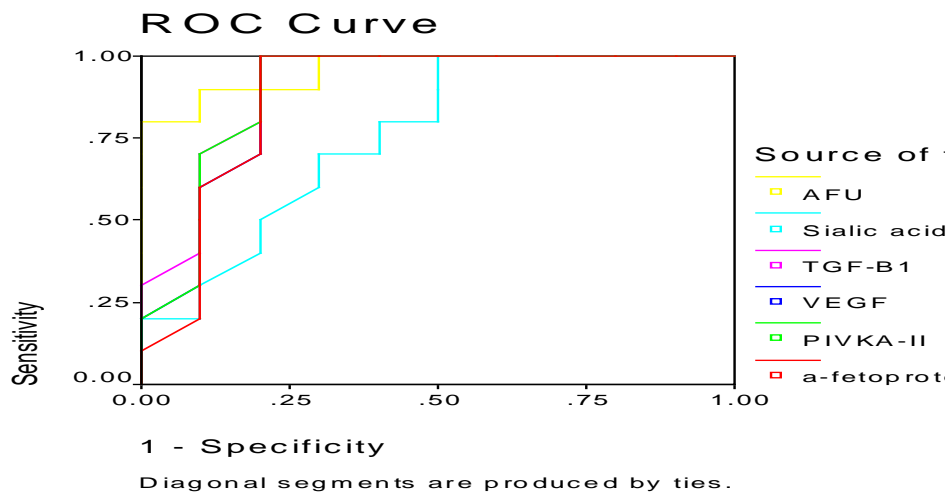


Figure 1: Multiple ROC curves for AFP, PIVKA-II, VEGF, Sialic acid, α-L-fucosidase (AFU) and TGF-β1

Table 6: The Cut-off value, sensitivity%, specificity%, PPV%, NPV%, accuracy% and AUC for AFP, PIVKA-II, TGF-β1, VEGF, sialic acid and AFU.

	Cut-off value	sensitivity %	specificity %	PPV%	NPV%	Accuracy %	AUC
AFP (ng/mL)	28 ng/mL	80%	97%	92.3%	86.6%	90%	0.880
PIVKA-II (mAU/mL)	12.5 mAU/mL	80%	97%	96%	86.9%	90%	0.900
TGF-β1 (ng/mL)	32.4 ng/mL	76.7%	97%	95.8%	84.7%	88.5%	0.910
VEGF (pg/mL)	780 pg/mL	96.7%	85%	82.4%	97.1%	90%	0.890
Sialic Acid (mg/dL)	90 mg/dL	80%	78%	72.7%	77.7%	75%	0.760
AFU (nmol/ml/h)	443 nmol/ml/h	80%	90%	81.8%	88.8%	85%	0.960

Table 7: Correlations between different parameters in hepatocellular carcinoma group (with and without metastasis).

	p-value	r- value
serum VEGF and plasma PIVKA-II	p = 0.012*	r = - 0.594
Serum AFP and alpha-L-fucosidase	P = 0.428	r = 0.283
Serum AFP and sialic acid	P = 0.692	r = 0.144
Serum AFP and VEGF	P = 0.322	r = 0.113
Serum AFP and TGF-β1	P = 0.106	r = 0.075
Serum AFP and PIVKA-II	P = 0.091	r = 0.027

*Significant

DISCUSSION

Hepatocellular carcinoma is one of the most prevalent causes of death in the world. Recently introduced therapeutic approaches for HCC, including percutaneous radiofrequency ablation (RF), transarterial chemoembolization and percutaneous ethanol injection (PEI) have improved the short term prognosis of small sized HCCs. To incorporate the benefit of such advancements into practice, early detection of hepatocellular carcinoma is essential¹⁸.

Because the majority of HCCs develop in cirrhotic livers, HCC surveillance with AFP and ultrasonography has been recommended for persons with cirrhosis. However, AFP level is insensitive in early diagnosis of the disease¹⁹. In addition, non specific elevation of serum AFP has been frequently found in patients with non malignant hepatocellular disorders such as acute hepatitis, chronic hepatitis, and liver cirrhosis⁹. Thus, new serologic markers with sufficient sensitivity and specificity are required for early detection of HCC.

In the current study, we aimed at evaluating the diagnostic and prognostic role of AFP, PIVKA-II, VEGF, sialic acid, AFU and TGF- β 1 in the diagnosis and follow up of patients with hepatocellular carcinoma in an attempt to find a tumor marker with a reasonable sensitivity and specificity for diagnosis of HCC and monitoring patients after therapy.

In the present study, there was a statistically significant difference on comparing the median level of serum

AFP in the HCC groups (with and without metastasis) with the control, the benign focal lesions and the cirrhotic groups (<0.001). Such significant difference between the controls, cirrhotic and HCC groups has been demonstrated in various studies^{18, 20-22}. Higher levels were detected in the metastatic HCC with significant difference between that group and the HCC group without metastasis ($p < 0.05$).

In the current study, the sensitivity of AFP was 80% and the specificity was 97% at the cut-off level of 28 ng/mL, PPV of the test was 92.3%, NPV was 86.6%, AUC was 0.880 and the overall accuracy was 90%. The sensitivity of AFP in the present study (80%) was higher than that reported by **Soresi et al.**²¹ who found that, at a cut-off value of 30 ng/ml, the sensitivity was 65% and the specificity was 89%, PPV 74% and NPV was 79%. Also, **He et al**²² reported that at a cut-off level of 25 ng/ml, the sensitivity was 68.7% and the specificity was 86.7%. The reported sensitivities and specificities in different studies could be attributed to the difference in the number of studied population and the methods used for assay.

Although the precise mechanism of plasma PIVKA-II production in HCC is not fully understood, it is widely accepted as a tumor marker for HCC in Japan¹⁸. It is reported that PIVKA-II is a significant prognostic factor for recurrent HCC^{23, 24}. Furthermore, combined elevation of tissue PIVKA-II expression and plasma PIVKA-II levels was found in cases with poor prognosis⁵. In our study, patients with HCC (group

III&IV) revealed a significantly higher level of PIVKA-II when compared to the other groups ($p < 0.001$). The median plasma PIVKA-II was found to be higher in the metastatic HCC patients than in those without metastasis ($p < 0.05$).

In the current study, PIVKA-II yielded an AUC of 0.900, at a cut-off level of 12.5 mAU/ml, the sensitivity was 80% and the specificity was 97%. In agreement with our finding, **Gotoh et al.**²⁵ who stated that PIVKA-II has a higher AUC than AFP in HCC diagnosis by the ROC analyses, while others²⁶ reported less AUC of 0.800. Different sensitivities and specificities of 62% and 95% and 53.3% and 85.6% were reported at a cut-off level of 40 mAU/ml, respectively^{27,28}. However, such difference may be attributed to selection of different cut-off value, variation in tumor size, number of masses or to difference in the number of studied cases.

In the present study, the median serum VEGF level was significantly higher in the HCC group without metastasis compared to the group with distant metastasis ($p < 0.001$). Similarly, **Zetter**²⁹ reported a high VEGF expression in small sized and well differentiated HCCs. He suggested that VEGF plays its most important role in a relatively early stage of the disease. In agreement with these results, **Mathonet et al.**³⁰ reported that VEGF level gradually decrease with increasing tumor size. **El-Assal et al.**³¹ reported that HCC nodules larger than 5cm in diameter were not more vascular than smaller tumors. On the other hand, **Tseng et al.**³² demonstrated that serum VEGF level in HCC patients showed a

positive correlation with tumor size. Furthermore, **Poon et al.**³³ reported that high serum level of VEGF was significantly associated with venous invasion and advanced tumor stage. Also, **Sun et al.**³⁴ and **Yao et al.**³⁵ showed that high expression of VEGF is a useful predictor of vascular invasion and metastasis of liver tumors. The discrepancy in serum VEGF level in the metastatic and non-metastatic HCC groups between our results and the others could be attributed to the different mass size. In our study the diameter of the HCC focal lesions in the metastatic group (group IV) was >6 cm, which may compress the blood vessels resulting in low serum VEGF level.

Transforming growth factor- β 1 (TGF- β 1) is a potent cytokine involved in a number of different functions such as epithelial mesenchymal transition, tissue morphogenesis, angiogenesis, and hence tumor progression, invasion, and metastasis. In liver tissues, TGF- β 1 has a profibrotic activity, stimulating extracellular matrix production, and is involved in the pathogenesis of liver cirrhosis. In other tissues, it stimulates integrin expression in different types of both normal and cancer cells³⁶. In the present study, the median plasma TGF- β 1 level was found to be significantly higher in the HCC groups when compared to control, benign and cirrhotic groups ($p < 0.001$). This in agreement with **Gianluigi et al.**³⁶ and **Anna. et al.**³⁷ who reported that increased secretion of TGF- β 1 by cancer cells is thought to facilitate tumor progression by indirect means, such as suppression of immune

surveillance and stimulation of tumor stroma.

Sialic acid, a class of important ketoses that contain nine carbon atoms, is an acetylated derivative of neuraminic acid (2-keto-5-amino-3, 5-dideoxy-D-nonulosonic acid). The unique structural features of this molecule, which includes a negative charge owing to a carboxyl group, enable it to play an important role in cellular functions, such as cell to cell recognition and transformation to malignancy¹⁰. As sialic acid lacked tumor specificity, it might contribute towards the early detection of tumor progression and metastasis during both therapy and follow-up. It was concluded that total serum sialic acid level reflected the development of malignancy and should be considered as a supportive evidence of being tumor marker for many malignant conditions¹⁰. In the present study, patients with malignancy revealed a significantly higher level of sialic acid when compared to the other groups ($p < 0.001$). The median serum sialic acid was found to be higher in the metastatic HCC patients than in those without metastasis ($p < 0.001$). This is in agreement with **Cai Wen-Xiu et al.**³⁸ who reported that most patients with HCC have an elevated concentration of serum total sialic acid, and determination of this marker yields high diagnostic values that differentiate between HCC and benign hepatobiliary diseases.

In the current study, the sensitivity of sialic acid was 80% and the specificity was 78 % at the cut-off level of 90 mg/dL, PPV of the test was 72.7%, NPV was 77.7%, AUC was 0.760 and the overall accuracy

was 75%. The sensitivity of sialic acid in our study was different from that reported by **Cai Wen-Xiu et al.**³⁸, who reported that at a cut-off value of 63 mg/dL, the sensitivity was 70 % and the specificity was 80%, PPV 75% and NPV was 80% and by using the cut-off value of 63 mg/dL, the obtained sensitivity and specificity were lower than that obtained by Cai Wen-Xiu et al.³⁸. The differences in the reported sensitivities and specificities could be attributed to the difference in the number of studied population and the methods used for assay.

Alpha-L-fucosidase (AFU) is enzyme belongs to the family of hydrolases, specifically those glycosidases that hydrolyse O- and S-glycosyl compounds. The systematic name of this enzyme class is alpha-L-fucoside fucohydrolase. It participates in N-glycan degradation and glycan structures - degradation.^{12, 39}

In the present study, patients with malignancy revealed a significantly higher level of AFU (group III and IV) when compared to the other groups ($p < 0.001$). the results of the present study were in agreement with **Deugnier et al.**¹¹ who reported a significant increase in serum α -L-fucosidase activity among patients with hepatocellular carcinoma as compared either to patients with cirrhosis or to healthy control subjects. The median serum AFU was found to be higher in the metastatic HCC patients than in those without metastasis ($p < 0.05$). Also, our results were in agreement with **Marotta et al.**⁴⁰ who stated that hepatocellular carcinoma is able to release into the blood stream a significant amount of

alpha-L-fucosidase, which correlates with the size of the tumor and provides a useful marker in the follow-up of patients treated for HCC.

In the present study, AFU yielded an AUC of 0.96, at a cut-off level of 443 nmol/mL/h, the sensitivity was 80%, the specificity was 90%, PPV was 81.8%, NPV was 88.8% and the accuracy was 85%. In agreement with our finding, **Giardina et al.**³⁹ who stated that When 443 nmol/ml/h is taken as the cut-off value; alpha-L-fucosidase sensitivity and specificity were 76% and 90.9%, respectively.

In the current study, a statistically significant lowering of the median blood levels of all parameters six months after ablation therapy was noticed. Similarly, **Ikoma et al.**⁴¹ reported that plasma PIVKA-II level was normalized in 67% of patients after treatment and that combined measurement of PIVKA-II with AFP is useful for monitoring recurrence of HCC after treatment. **Sheen et al.**⁴² found that VEGF is a significant biological indicator of the invasiveness of postoperative recurrence. Also, **Cai Wen-Xiu et al.**³⁸ reported that measurement of sialic acid is useful for monitoring recurrence of HCC after treatment.

In the current study a statistically significant negative correlation was found between serum VEGF and plasma PIVKA-II in the HCC groups (with and without metastasis) (Table 7). Such correlation was not found between serum AFP and the other markers. No correlation was found between AFP level and alpha-L-fucosidase activity, AFP and sialic acid level, AFP and VEGF, AFP and PIVKA-II and AFP and TGF- β 1 (p

=0.428, 0.692, 0.322, 0.091 and 0.106 respectively) (Table 7). These findings confirm those of **Ishii et al.**¹⁸ and **Cui et al.**²⁸ who found no correlation between AFP level and PIVKA-II level in the HCC patients and **Shirai et al.**⁴³ who could not find a significant correlation between TGF- β 1 and AFP level in HCC patients. Also, serum AFP and AFU activity were independent with no correlations between them as evidenced by **Cai Wen-Xiu et al.**³⁸.

In the present study, combination of two markers or more improved both sensitivity and specificity as combination of AFP and PIVKA-II yielded a sensitivity and specificity of 93.3% and 98.2% and for AFP with TGF- β 1, the sensitivity and specificity were 87.5% and 99%, respectively. By combined measurement of AFP and AFU, the sensitivity and specificity were 95% and 98%, respectively. While combined measurement of both AFP and sialic acid gave sensitivity 89 and specificity 98% as three of the twenty patients with HCC who had AFP level below the cut-off value, had positive results in AFU and another one had a positive result in sialic acid. Therefore, combined measurement of serum AFP and AFU, AFP and PIVKA-II, AFP with TGF- β 1 or AFP and sialic acid is of practical significance in diagnosis of hepatocellular carcinoma.

In the current study, the main purpose of using combined measurement of tumor markers is to eliminate the false negative and false positive results as reported by many authors^{10,18}. After reviewing our results as well as those of others, the

question on the usefulness of combining markers in assessing early diagnosis of malignant liver disease is raised. Our data support the highly significant value of combined markers as a screening tool for early diagnosis in susceptible individuals. Therefore, combined determination of the studied serological markers could be used as a highly valuable tool for screening and diagnosis of hepatocellular carcinoma. They could also be used as prognostic markers decreasing the need for more invasive procedures. The combined use of easy and cheap serological markers with sufficient sensitivity and specificity is of great value in follow up of HCC patients as by combined measurement of α -fetoprotein and α -L-fucosidase or α -fetoprotein and sialic acid. Also, according to our results these markers offer a potential for using them for follow up after treatment.

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القيمة التشخيصية والمندرة لبعض دلالات الاورام في تشخيص ومتابعة مرضي سرطان الكبد

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المقدمة والهدف من البحث:

يعد مرض سرطان الكبد من اخطر انواع الاورام واكثرها شيوعا وتنشأ خطورته من تأخر اكتشافه واكتشاف الحالات في مرحلة متأخرة يصعب تلافي اثارها ومن ثم كان الاكتشاف المبكر للمرض سببا هاما في خفض نسبة الوفيات لهذا المرض وهذا استوجب البحث عن بعض دلالات الاورام والتي لديها حساسية وخصوصية مرتفعة الي حد يساهم في الكشف المبكر عن اغلب الحالات والتي بواسطتها يمكن تشخيص ومتابعة المرضي اثناء العلاج. وفي هذا البحث هدفنا الي تقييم دور كل من حمض السياليك، نشاط انزيم الفا-ال-فيوكوسيديز، عامل النمو المحول-بيتا 1، عامل النمو المبطن للاوعية، البروتين الناتج عن غياب فيتامين ك في

الدم وذلك في تشخيص ومتابعة مرضي سرطان الكبد سواء في المراحل الاولية للمرض قبل العلاج او في بعض المصابين بورم سرطاني بالكبد يصاحبه انتشار للمرض باماكن اخري بالجسم ومقارنة نتائج ابحاث الدلالات السابق ذكرها لهؤلاء المرضى بنتائج الاشخاص الطبيعيين ونتائج مرضي التشمع الكبدي والمرضى المصابين ببعض الاورام الحميدة ومقارنة النتائج الخاصة بها والتي حصلنا عليها من خلال هذا البحث بنتائج الفا فيتو بروتين في دم نفس المرضى والتي حصلنا عليها في ذات البحث والذي يعد كواحد من اهم دلالات الاورام المستخدم حاليا في تشخيص ومتابعة حالات اورام الكبد علي الرغم من ان درجة حساسيته لا تتجاوز 75% والتي تعني قدرته علي كشف ثلاثة ارباع المرضى فقط كما ثبت بالابحاث العلمية السابقة. كما تم متابعة مجموعة المرضى المصابين باورام الكبد اولى ولايصاحبها انتشار للورم لاماكن اخري بالجسم والذين تم معالجتهم بطرق غير جراحية قبل وبعد العلاج بستة اشهر لاختبار مدي قدرة هذه الدلالات علي متابعة المرض وقدرتها علي الانذار المبكر عند حدوث أي مضاعفات.

مواد البحث:

تم إجراء هذا البحث على خمسين شخصا من الجنسين وتم تقسيمهم إلى خمس مجموعات: المجموعة الضابطة وعدد الأشخاص بها 10 أشخاص وهم اشخاص طبيعيين واربع مجموعات من المرضى مقسمة الي : المجموعة الاولي ومكونة من عشرة مرضي مصابين بالتشمع الكبدي ، المجموعة الثانية مكونة من عشرة مرضي مصابين بانواع مختلفة من الاورام الحميدة ، المجموعة الثالثة مكونة من عشرة مرضي مصابين باورام كبدية خبيثة اولى ولايصاحبها اورام ثانوية والمجموعة الرابعة مكونة من عشرة مرضي مصابين باورام كبدية خبيثة اولى و يصاحبها اورام ثانوية ولم يكن هناك اختلاف ذو دلالة احصائية في متوسط العمر ونوع الجنس بين المجموعات الخمسة. وقد تم علاج المرضى في المجموعة الثالثة (المرضى المصابين باورام سرطانية بالكبد ولا يصاحبها انتشار للمرض باماكن اخري بالجسم) ببعض الطرق الغير جراحية ثم تبع ذلك متابعة المرضى بقياس نفس دلالات الاورام السابقة بعد العلاج بستة اشهر وبعد التأكد من تلاشي الورم باستخدام الأشعة المقطعية.

طرق البحث:

تم أخذ التاريخ المرضي للمرضي المدرجين تحت هذه الدراسة وتم فحصهم فحصاً إكلينيكياً كاملاً وتم عمل الأبحاث المعملية التالية لكل مجموعات المرضى وايضا للمجموعة الضابطة: مستوي كل من الفا - فيتو بروتين ، حمض السيليك ، نشاط انزيم الفا -ال-فيوكوسيديز ، عامل النمو المحول-بيتا-1 ، عامل النمو المبطن للاوعية والبروتين الناتج عن غياب فيتامين ك في الدم . كما تم عمل عينة كبدية للمرضي للفحص والتشخيص الهيستوباثولوجي .

نتائج البحث:

قد وجد فروق ذات دلالة احصائية في مستوي جميع الدلالات المقاسة بين المرضى المصابين باورام خبيثة بالكبد (مجموعة 3، مجموعة 4) وبين المجموعة الضابطة وبقية المرضى كما وجد فروق ذات دلالة احصائية بين المجموعة الثالثة المصابين باورام خبيثة بالكبد ولا يصاحبها بور ثانوية قبل وبعد العلاج مما يدل علي امكانية استخدام جميع الدلالات الكيميائية المقاسة كدلالات لاورام الكبد مع اختلاف درجة حساسيتها ودرجة تقييمها للتنبؤي بالمرض .

اشار البحث ايضا الي امكانية استخدام دلالتين او اكثر لتشخيص حالات اورام الكبد الخبيثة والذي يرفع من درجة الحساسية والخصوصية لتلك الدلالات ويقلل من نسبة المرضى الذين لا يتم تشخيصهم بسبب انخفاض نسبة الحساسية والخصوصية للدلالات الكيميائية المستخدمة وبالتالي فان استخدام اكثر من دلالة كيميائية لكل مريض يساهم في الكشف والتشخيص المبكر للحالات مما يؤدي الي تحسين الحالة المستقبلية للمرضي ويزيد من الفترة التي يحياها المريض في سلام .