ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

PIVKA-II AS EARLY DIAGNOSTIC MARKER FOR HEPATO CELLULAR CARCINOMA: AN EGYPTIAN STUDY

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Manuscript Info

Manuscript History:

Received: 11 October 2013
Final Accepted: 22 October 2013
Published Online: November 2013

Key words:
HCC; prothrombin induced by vitamin K absence-II; Interleukin-8.

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Abstract

Hepatocellular carcinoma (HCC) is one of the most aggressive cancers worldwide. In Egypt, the disease is detected in an advanced stage at which no treatment may be effective including surgery. Among Egyptians, viral hepatitis is the most common risk factor for HCC. Early detection of the disease is thus an important goal allowing the patient to be treated before the enlargement of the tumor or its metastasis to distant organs. Serum tumor markers may be useful in predicting the tumor at early stages. The current work aimed to determine the level of prothrombin induced by vitamin K absence-II (PIVKA-II) and Interleukin – 8 (IL -8) in sera of patients suffering from HCC & cirrhosis and comparing which one is more sensitive and specific for early diagnosis of HCC. The current study was carried out on 150 individuals within three groups; Normal control, Cirrhosis and HCC groups. Complete examination was carried out for each individual to confirm diagnosis. Individuals' sera were subjected to quantitative determination of alpha-fetoprotein (AFP), TNF, PIVKA-II, Il-8 and other biochemical parameters. PIVKA-II was proved to be superior to AFP, TNF and IL-8 for early detection of HCC patients being highly sensitive and specific. Using the best cut-off value of AFP (6.5), showed a sensitivity of (88 %) and specificity of (60 %) and IL-8 cut-off value (128.5) , showed a sensitivity of (96 %)and specificity of (99%), While cut-off value of PIVKA-II (9.45) showed (100%) sensitivity and specificity (99%).

Introduction

HCC is the seventh most common cancer worldwide, and the third leading cause of cancer-related deaths. In 2008, an estimated 748,000 new cases of liver cancer occurred and approximately 696,000 people died of this cancer worldwide, an increase from 626,000 new liver cancers and 598,000 deaths from liver cancer in 2002**[1].** The etiology of HCC differs according to geographic, economic, and health status. HCC ranks number one with an incidence rate of 70.48% **[2].** The most common causes are alcohol consumption **[3]**, hepatitis C and B viruses **[4]** and chronic necro- inflammatory hepatic disease. Commonly cirrhosis is present in 60–80% of patients with HCC **[5].** Among Egyptian patients HCV and HBV infections are the most common risk factors for HCC. About 10% – 20% of the general Egyptian population is infected with HCV **[6].** So, these made essential to find sensitive markers for early diagnosis and monitoring of recurrence of HCC **[7].** Ultrasound examination of the liver and detection of AFP level in serum are commonly used to screen for liver cancer **[8].** Although detection of AFP level is easy and less expensive, but it shows less sensitivity **[9],** since elevation in AFP level is common in patients with chronic liver disease, pregnancy and germ cell tumors. AFP titers also rise with flares of active hepatitis, and may be persistently elevated in patients with cirrhosis **[10].** Prothrombin induced by vitamin K absence-II (PIVKA-II) is also known as

Des- gamma carboxyprothrombin (DCP) is an abnormal prothrombin protein that is increased in the sera of patients with HCC. Generation of (PIVKA-II) is thought to be a result of an acquired defect in the post-translational carboxylation of the prothrombin precursor in malignant cells **[11].** The validity of PIVKA-II as a tumor marker for HCC patients has been reported by many investigators **[12- 13].** PIVKA-Ⅱ is an abnormal protein and some reports have indicated the improved specificity of PIVKA-Ⅱ over AFP in the diagnosis of HCC[2,5,27-29]

None of the known markers are optimal, however when used together their sensitivity increases **[14-15].** IL-8 is a multifunctional CXC chemokine that affects human neutrophil functions, including chemotaxis, enzyme release, and expression of surface adhesion molecules. IL-8 is produced by a wide variety of cell types, including monocytes, neutrophils, fibroblasts, and endothelial cells [16–17]. IL-8 was identified to be an angiogenesis-regulating molecule that induces angiogenesis. The expression of IL-8 has been found in various human cancers [18]. Various studies have demonstrated that IL-8 regulates tumor cell growth and metastasis in melanoma [19], carcinoma of breast [20], stomach, pancreas and liver [21-22]. Akiba et al [22], provided evidence that IL-8 produced by HCC is an angiogenic factor of HCC. Therefore, it is of interest to elucidate the role of serum IL-8 as a biological tumor marker in HCC patients. Very little work was done to clarify the role of IL-8 in early detection of HCC in Egyptian patients. The present study was designed to investigate the potential role of PIVKA-II and IL-8 as a diagnostic marker for HCC at their early detection and to assess their sensitivity and specificity as compared with the usual recommended marker AFP.

Patients and methods

This study was conducted on 150 patients, divided into 3 groups. 50 patients as cirrhosis group , 50 patients as HCC group and 50 apparently healthy individuals as control. An informed consent was taken from each patients before the beginning of the study. Also, the study was approved by the ethical committee of Faculty of medicine. Patients were initially subjected to complete clinical examination and abdominal ultrasonography. Blood samples were collected for complete blood picture, liver function tests, and fasting blood sugar levels using the standard laboratory methods. Hepatitis markers HBsAg, HCV-Ab were detected using ELISA technique, HCV RNA by qualitative PCR. Serum level of AFP was detected using ELISA technique (Monobind INC. kit USA) , PIVKA-II level using ELISA kit (Sanko Junyako – Tokyo) and IL-8 was detected using ELISA technique (Quantikine Human CXCL8/IL-8 kit, USA) .

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences, Version 17.0 (SPSS, Inc., Chicago, III., USA) for Windows. Continuous variables were analyzed as mean values ± standard deviation (SD) or median (range) as appropriate. Rates and proportions were calculated for categorical data. For categorical variables, differences were analyzed with $\chi 2$ (chi square) tests and Fisher's exact test when appropriate. Differences among the three groups regarding continuous variables with normal distribution) were analyzed with Univariate ANOVA and Bonferroni post hoc test and that not normally distributed was done by kruskel walli test. Receiver Operator Curve (ROC curve) was done to determine the cut off value of different markers in diagnosis of HCC. Correlations were determined by using Pearson's test. P value of $\leq$ 0.05 was considered statistically significant

Results:

In the present study, there was no statistical significant difference between the three studied groups as regards age and sex. Meanwhile sex distribution showed that HCC patients was more common in males than females (Table 1) .

Table (2) showed highly significant difference of AST, ALT, T.bili., D.bili., INR, and GGT levels in HCC and cirrhotic patients groups compared to healthy control ($p <$ **0.001).** On the other hand, AST, ALT, T.bili., D.bili., INR and GGT were highly significant increase in HCC group compared to cirrhotic group. There was a higher mean level of IL-8, Pivka-II, , AFP and TNF-α among cases with HCC compared to cirrhosis and control groups with highly statistically significant difference (Table 2).

In the present study, statistical analysis showed that hepatic focal lesions that diagnosed by computed tomography were rounded in (58%), more frequent in right lobe (72%) (Table 3). As regards the characteristics of the studied groups, there were no statistical difference between HCC and cirrhosis concerning weight loss, elevation of temperature, abdominal pain and jaundice. Meanwhile, encephalopathy and bleeding showed high statistical significant difference between the 2 groups (Table 4).

Table (5) showed the relation between the serological parameters IL-8, Pivka-II, AFP and TNF-α and to tumor size in HCC group. The four markers showed no statically significant difference with tumor size (Table 5).

Table (6) showed correlation between the four parameters measured in the serum of HCC group according to the roc curve and the area under the curve. The area under the curve for pivka-II was the best tumor markers with high sensitivity (100%) and high specificity (99%) (Figure 1).

Table (1): Demographic data of the three studied groups:

Parameters	HCC n=50(%)	Cirrhosis n=50(%)	Control n=50(%)	P value
Age (yrs)				
Mean± SD	58.9±9.7	58.2±10.9	55.9±13.9	0.589(>0.05)
Gender				
Male	31(62.0)	27(57.0)	9(45.0)	0.408(>0.05)
Female	19(38.0)	23(43.0)	11(55.0)	

P > 0.05 = non significant

Table (2): Comparison of laboratory investigations of individuals in the three groups.

Laboratory Results	HCC N=50(%) Mean± SD	Cirrhosis n=50(%) Mean± SD	Control n=50(%) Mean± SD	P value
AST	149.7±66.2	57.9±29.0	32.4±9.1	<0.001
ALT	64.3±18.9	57.2±16.5	30.0±6.0	<0.001
T.Bilirubin	2.7±1.0	1.3±0.6	0.7±0.2	<0.001
D.Bilirubin	0.8±0.4	0.3±0.2	0.2±0.1	<0.001
Albumin	2.7±0.6	2.7±0.7	3.9±0.2	<0.001
INR	2.1±0.3	1.9±2.1	1.1±0.1	<0.001
GGT	224.3±127.1	46.3±23.2	34.3±9.5	<0.001
IL-8	242.7±162.0	140.4±36.5	97.2±4.6	<0.001
Pivka-II	33.1±12.9	19.6 ±5.9	0.7±0.3	<0.001
AFP	306.8±281.5	35.1±33.7	5.9±2.0	<0.001
TNF-α	149.18 ±45.8	138.62 ±32.5	103.85 ±7.6	<0.001

P < 0.001 = highly significant

Table (3): Descriptive analysis of hepatic focal lesions by computed tomography

	NO	%
Shape		
Oval	21	42.0
Rounded	29	58.0
Site		
Lt.lobe	14	28.0
Rt .lobe	36	72.0

Table (4): Clinical characteristics of the diseased groups:

	HCC n=50(%)	Cirrhosis n=50(%)	P value
Weight loss			
Yes	21(42.0)	22(44.0)	0.840
Hyperthermia			
Yes	20(40.0)	23(46.0)	0.527
Abdominal pain			
Yes	25(50.0)	21(42.0)	0.422
Jaundice			
Yes	45(90.0)	47(94.0)	0.715
Encephalopathy			
Yes	33(66.0)	12(24.0)	0.000
Bleeding			
Yes	30(60.0)	18(36.0)	0.016

P < 0.001 = highly significant

Table (5): Comparison between IL-8, Pivka_II , AFP and TNF α with tumor size in HCC group:

Parameters	Tumor sizes Mean± SD <3 n (9)	3-5 n (20)	>5 n (21)	P value
IL-8	278.88 ± 212.21	245.45 ±166.97	224.62±137.29	0.707
Pivka-II	31.0± 13.49	37.7± 13.24	29.53 ± 11.46	0.110
AFP	502.22± 308.45	274.25± 297.63	254.09 ± 225.24	0.066
TNFα	147.22± 44.08	178.75± 41.71	121.85± 32.24	0.075

P > 0.05= non significant

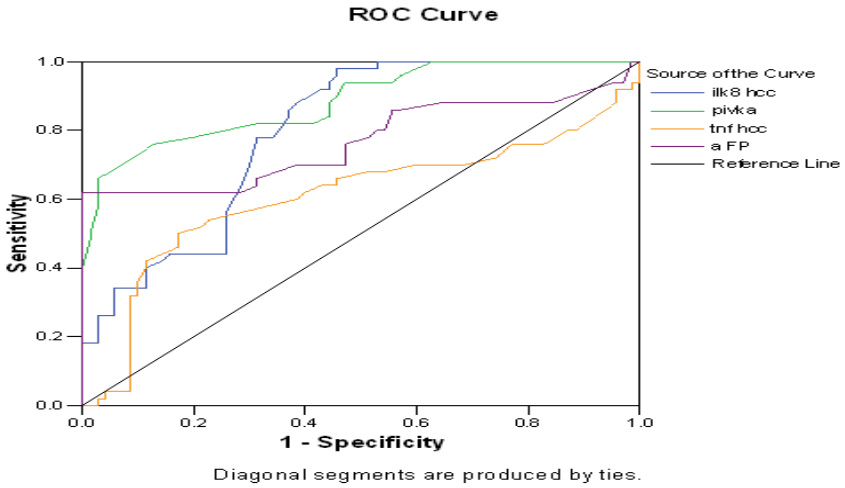
Table (6): Correlation between the three markers according to the Roc Curve and the Area under the Curve:

	Area	P- value	Cut off	Sensitivity %	Specificity %
IL-8	**.796**	**<0.001**	**128.5**	**96**	**99**
PIVKA-II	**.884**	**<0.001**	**9.45**	**100**	**99**
AFP	**.767**	**<0.001**	**6.5**	**88**	**60**
TNF-α	**.432**	**<0.23**	**124.5**	**58**	**64**

Discussion

HCC is a leading cause of mortality among patients with cirrhosis**[23].** Detection of HCC at early stages is critical for good clinical outcome as the prognosis of HCC patients is very poor when diagnosed at late stages **[24-25].** Although serum AFP is the most established tumor marker in HCC and considered as the golden standard to which

other markers are compared, it was found to be normal in about 30% of the patients, especially in early stages **[26-27].** Elevated levels might be seen in patients with cirrhosis or exacerbation of chronic hepatitis **[27-28].** Therefore there is an increased need for new tumor markers that may be more sensitive and specific for HCC. **In this study,** we didn't find any significant difference between HCC patients compared to either to cirrhosis patients or control patients as regards to age. In HCC patients the age ranged from 35-76 years with mean age of incidence 58.8 years old. These results were agreed with **El zayadi et *al* [29],** who reported that analysis of age distribution among HCC patients revealed that the most predominant age group was (40-59 years). Also, in the present study, HCC patients were more common in males than females, these results are similar to **Zakhary et al [30]** who reported that males represented 70.8% of all patients in HCC group, with 83.3% of patients over 50 years. Meanwhile, our results disagreed with **Levrero [31]** who reported that, HCC was the fifth most common cancer in males and the eighth common cancer in females and about 560.000 cases were discovered per year , more than 80% of which occur in the developing countries . **In our study** we found that the mean value of AST, ALT , Bilirubin , INR and GGT were higher in HCC patients , while albumin was lower in HCC group. These findings are consistent with **Ahmed [32]** who reported that the previous parameters usually indicate the type of liver injury, whether hepatocellular or cholestatic, but cannot be expected to differentiate one form of hepatitis from another or to determine whether cholestasis is intra or extra hepatic.



The present study revealed a significant elevated level of IL-8, PIVKA-II, AFP and TNF-α in cases of HCC. These findings was agreed partially with **Ibrahim et al [33]** who reported that serum IL-8 in HCC patients was elevated significantly as compared to control subjects. Also, our results coincided partially with **Zakhary et al [30]** who revealed a significant elevation of PIVKA-II and AFP levels in HCC group compared to control and HCV groups. PIVKA-II showed more increase than AFP level in malignant compared to benign liver diseases. **The present work** showed that no statistically significant difference between PIVIKA II and tumor size. This result coincided with that found by ***Sassa et al* [34]** *who* reported that there was no correlation of DCP to tumor size and type. Also, **Kanke et al [35]** found no significant relationship between DCP and AFP with respect to tumor size.

Meanwhile, our results disagreed with **Zakhary et al [30]** who reported that plasma PIVKA-II level was increased in correlation with tumor size to reach its maximum level for tumors with sizes more than 5 cm. At the same time, IL-8, AFP and TNF-α had no significant difference with tumor size. ROC curve was drawn to compare between the four markers to determine the best cutoff value. The results revealed that, the best cut-off value of IL-8(128.5) showed sensitivity (96%) and specificity (99%), PIVKA-II (9.45) showed sensitivity (100%) and specificity (99%), AFP (6.5) showed sensitivity (88%) and specificity (60%), while for TNF-α (124.5) showed sensitivity (58%) and specificity (64%).

In a meta-analysis based on literature review of 20 publications, the overall sensitivity, specificity of DCP was 67% (95%CI, 58–74%), 92% (95%CI, 88–94%) respectively. [36] In a study conducted by Bertino et al. serum DCP was found to have a sensitivity ranging from 48% to 62%, a specificity of 81–98%. [37], these variations may be attributed to variation of the sample size, tumor size or number of masses in different studies.

In conclusion the present study reveals that the high sensitivity and specificity of PIVKA-II may give value in screening high risk population and diagnose the disease at early stages when curative treatments are possible.

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