Detection of Serum Kl-6 as a Tumor Marker in Hepatocellular Carcinoma

[1]Soha Z. El-Shenawy, [2]Maha M El-Sabawy, [3]Eman Abd El-Razik, [4]Maha M Allam

Departments of [1]Clinical Biochemistry, [2]Hepatology and [4]Clinical Pathology, National Liver Institute, Menofiya University, Department of [3]Oncology, Faculty of Medicine, Menofiya University, Egypt
E-mail: sohazaki69@yahoo.com

ABSTRACT: Background: Hepatocellular carcinoma (HCC) is a common malignancy affecting approximately one million of people around the world every year and represents the fifth most common cancer worldwide. Early detection of the onset of HCC would help to select more effective therapies for patients, leading to a better prognosis and longer life span. **The aim of this study** was to evaluate the efficacy of KL-6 as a diagnostic marker of HCC in Egyptian patients. **Subjects & methods:** this study was conducted on three groups. Group 1: included 57 patients (48 males and 9 females) were diagnosed as HCC by the presence of characteristic hepatic masses on abdominal MRI, CT and/or hepatic angiography. Group 2: included 46 patients (37 males and 9 females) hepatitis B virus and/or hepatitis C virus –related cirrhotic patients. Group 3: included 40 subjects (32 males and 8 females) apparently healthy as a control group with no evidence of liver disease and/or neoplasm. Serum levels of AFP and KL-6 were measured in all patients groups and control groups. **Results:** A highly statistically significant difference was found between the three groups regarding the mean serum levels of both AFP and KL-6 where the highest increase of both markers were found in the HCC group. When Spesrman's correlation coefficient was done in the patients, a significant positive correlation (*P* value <0.001) of serum levels of AFP & KL-6 was found. When analysis of the results of AFP in the patients with HCC, this study found 39 patients (68.4%) having + ve AFP level (high AFP than its normal range), while 48 HCC patients (84.2%) are + ve for KL-6 level (high KL-6 than its normal range). In patients with liver cirrhosis, 19 (41.3%) patients have + ve AFP while 24 cirrhotic patients (52.2%) are + ve for KL-6 level. Results of ROC curves analysis show that the optimal cut-off values were 437 U/ml for KL-6 (sensitivity = 91.7% & specificity = 85.7%) and 102 IU/ml for AFP with sensitivity = 84.6% and specificity = 89.4%. **Conclusion:** These results suggest that KL-6 could be a promising tumor marker for early detection of HCC in Egyptian patients. A large scale study is needed to investigate its clinical usefulness in screening cirrhotic patient for HCC.

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1. Introduction

Hepatocellular carcinoma (HCC) accounts for 80% to 90% of primary liver cancer. HCC is a major health problem worldwide. It is the fifth most common cancer in the world and the third most common cause of cancer-related death. The rates of HCC in men are 2 to 4 times higher than in women. It usually develops between 35 and 65 years of age, when people are in their most productive era in their life with the outmost family responsibilities [(1)]. Complex carcinogenesis of HCC is a multi-factorial, multi-step and complex process, which is associated with a background of chronic and persistent infection of hepatitis B virus (HBV) and hepatitis C virus (HCV) [(2)].

HCC with poor prognosis has many characteristics, such as fast infiltrating growth, metastasis in early stage, high-grade malignancy and poorly therapeutic efficacy. Early detection may offer hope for a more favorable prognosis as most of HCC patients died quickly due to the rapid tumor progression [(3)].

Current diagnosis of HCC relies on clinical information, liver imaging and measurement of serum alpha-fetoprotein (AFP) [(4)]. Serum alpha-fetoprotein (AFP) was first described as a marker for HCC by **Abelev *et al.,*** in the **1960** and used as a serum marker for HCC in humans for many years [(5)]. The first quantitative serum assays for AFP were established by **Ruoshlati and Seppala** [(6)]. It has a sensitivity of 39%–65%, a specificity of 76%–94%, and a positive predictive value of 9%–50% [(7)]. HCC patients with a high AFP concentration (≥ 400 IU/mL) tend to have greater tumor size, bilobar involvement, massive or diffuse types, portal vein thrombosis and a lower median survival rate [(8)]. Though the measurement of AFP serves as an important tool in screening of HCC, some reports have indicated that it has limited utility of differentiating HCC from benign hepatic disorders for: its high false-positive and false-negative rates,

elevated levels in patients with acute exacerbation of viral hepatitis and that tumors other than HCC may also have markedly increased AFP levels like testicular tumors [8].

AFP with its reported sensitivity and specificity are not sufficient for early diagnosis as AFP concentrations are directly correlated with tumor size. So, the development of effective marker for the diagnosis of HCC could have an impact on HCC-related cancer mortality and significant public health implications worldwide [9].

A number of serum markers have been proposed and currently used as an effective method for detecting HCC long time ago. The most urgent need was to find sensitive markers for early diagnosis and monitoring of postoperative recurrence of HCC patients, to give adequate treatment for HCC patients [10].

Mucins are large glycoproteins with high carbohydrate content and marked diversity both in the apoprotein and in the oligosaccharide moieties [11]. MUC1 mucin, one kind of mucin glycoprotein, is abundantly expressed at the surface of epithelial cells in many tissues [12]. It seems to influence various physiological or biochemical events as diminished immune response and regulate cell adhesion properties [13].

KL-6 mucin is a type of MUC1 mucin, recognized by a murine monoclonal antibody (mAb) **Kohno *et al.* (1988)** [14]. Biochemical analyses displayed that the molecular weight of kL-6 mucin was over 200 kDa because of a large amount of carbohydrate content [15].

KL-6 has been first shown to be elevated in patients with interstitial pneumonia [16]. Also, serum KL-6 is a sensitive effective marker of disease activity in fibrosing lung diseases [17]. **Hirasawa *et al.*, 1997** [18] reported in the epithelial lining fluid in small airways may cause the intra-alveolar fibrosis in fibrosing lung disease that KL-6 is one of the chemotactic factors for most fibroblasts and that the increased KL-6 in the epithelial lining fluid in small airways may cause the intra-alveolar fibrosis in fibrosing lung disease. Many investigations have shown that aberrant expression of MUC1 in gastrointestinal cancer tissue has clinicopathological and biological importance in cancer disease [19]. KL-6 mucin, was also investigated and suggested to have a significant relationship with tumor behavior especially cancer cell invasion in various gastrointestinal and primary liver cancers [20].

It was also reported to have a high positive rate in different non-hepatic malignancies and its expression was also correlated with metastatic potential of the primary tumor in some of them [21]. It has also been studied as a fibrosis marker in patients with HCV-related chronic liver disease and was found to correlate with the degree of irregular regeneration of hepatocytes. A recent study addressed its clinical significance as a tumor marker in HCV-related HCC [22]. However, all these studies investigated KL-6 in HCV-related disease only so that its actual significance as a marker for screening HCC in patients with different chronic liver disease is not yet fully understood [22]. In the light of these observations, we studied both KL-6 and AFP serum titers in a cohort of HCC patients, as well as in patients with cirrhotic liver disease, and in healthy controls. This study aims to show the efficacy of KL-6 as a more specific, sensitive and accurate biomarker than AFP to help in early diagnosis of hepatocellular carcinoma.

2.Subjects & Methods

In this study, the patients were selected from the Department of Hepatology, National Liver Institute, Minoufiya University and Department of Oncology, Faculty of Medicine, Minoufiya University. There were three groups, group (1) included 57 patients (48 males and 9 females & their mean age was 46.87±6.58 years) were diagnosed as HCC by the presence of characteristic hepatic masses on liver MRI, CT and/or hepatic angiography (i.e., enlarged tumors and/or tumors with typical arterial vascularization), group(2) [liver cirrhosis (LC)] included 46 patients (37 males and 9 females & their mean age was 42.28±9.34 years) hepatitis B virus and/or hepatitis C virus –related cirrhotic patients. A third group included 40 patients (32 males and 8 females) apparently healthy subjects as a control group with no evidence of liver disease and/or neoplasm & their mean age was 40.9±8.69 years. All the procedures used in this study were approved by the Research Ethics Committee of National Liver Institute and Faculty of Medicine, Minoufiya University, Egypt. An informed consent was obtained from all subjects in this study.

All individuals included in this study were fasting overnight. 10 ml of venous blood were withdrawn by venipuncture in the morning. It was centrifuged and the serum was frozen for subsequent analysis.

Assessment of serum AFP was performed using VIDAS instrument, BioMerieux, France using the Enzyme Linked Fluorescent Assay (ELFA). The results were expressed as IU/ml.

Serum level of KL-6 was determined, by the sandwich enzyme immunoassay method using the KL-6 antibody (Ab) as both the capture and tracer Ab, using AviBion Human KL-6 ELISA Kit, Orgenium Laboratories, Finland [23]. The results were expressed as U/ml.

Statistical analysis: Statistics were carried out using Statistical Package for Social Science program (SPSS). Data were presented as mean ± SD. Kruskal Wallis test (non parametric test) used for the values which aren't normally distributed in order to compare more than 2 groups. Determination of spearman's correlation coefficient (r) was done for correlation between serum AFP & KL-6 as a quantitative variables. Receiver Operating Characteristic (ROC) curve was produced for the measured parameters to investigate the sensitivity, specificity and the cut-off values of each AFP and KL-6. P value less than 0.05 was considered statistically significant.

3. Results

Table (1) showed the patient's background in the three studied groups regarding the presence of viral infection (either HCV, HBV, both HCV-HBV and none), Child classification and tumor size & its differentiation.

When analysis of variants were done between the three groups, a highly statistical significant difference was found between these groups regarding the mean serum levels of both AFP and KL-6 where the highest increase of both markers were found in the HCC group (Table 2). When Spesrman's correlation coefficient was done in the patients, a significant positive correlation (P value <0.001) of serum levels of AFP & KL-6 was found (Table 3).

On analysis of the results of AFP in the patients with HCC (Table 4), 39 (68.4%) patients are + ve AFP level (high AFP than its normal range), while 48 HCC patients (84.2%) are + ve for KL-6 level (high KL-6 than its normal range). In the patients with liver cirrhosis, 19 (41.3%) patients have +ve AFP while 24 patients with cirrhosis are + ve for KL-6 serum levels. As regards KL-6 serum levels in relation to Child classification in both patient groups with liver cirrhosis (103 patients), a positive correlation was detected between the patients, with a progressive increase in serum levels of KL-6 from patients with child A cirrhosis (424.5 U/ml), to patients with child B cirrhosis (1086 U/ml), up to patients with child C cirrhosis (3799 U/ml) (Table 5).

Table (6) illustrates another positive correlation which was found between the KL-6 serum levels in relation to the tumor size in the HCC group (57 patients). KL-6 was found to be (424.5 U/ml) in patients with tumor size less than 3 cm, (518.7 U/ml) in patients with tumor size 3-5 cm, and (752.3 U/ml) in patients with tumor size 5 cm.

Figure (1) represent the ROC curve of KL-6 as a test variable in comparison with AFP in the patients and figure (2) represent the ROC curve of AFP as a test variable in comparison with KL-6 in the same patient groups. Results of ROC curves analysis show that the optimal cut-off values were 437 U/ml for KL-6 (sensitivity = 91.7% & specificity = 85.7%) and 102 IU/ml for AFP with sensitivity = 84.6% and specificity = 89.4% as reported in table (7).

Table (1): Characteristics of the groups

	HCC group (N=57)		LC group (N=46)		Control group (N=40)	
	No	%	No	%	No	%
Gender: Male	48	84.2	37	80.4	32	80
Female	9	15.8	9	19.6	8	20
Viral infection:						
HCV-related	26	45.6	24	52.2	18	45
HBV-related	3	5.3	2	4.4	2	5
Both C&B-related	9	15.8	6	13	3	7.5
Non-viral	19	33.3	14	30.4	17	42.5
Child classification:						
A	12	21.1	16	34.8	00	00
B	13	22.8	11	23.9	00	00
C	32	56.1	19	41.3	00	00
Tumor size:						
< 3 cm	15	26.3	00	00	00	00
3-5 cm	25	43.9	00	00	00	00
>5 cm	17	29.8	00	00	00	00
Tumor multiplicity:						
Solitary	33	57.9	00	00	00	00
Multiple	24	42.1	00	00	00	00
Differentiation:						
Well	39	68.4	00	00	00	00
Poor	18	31.6	00	00	00	00

Table (2): AFP and KL-6 serum levels in the all studied groups

	HCC group (N=57) Mean ± SD	LC group (N=46) Mean ± SD	Control group (N=40) Mean ± SD	Test of sign. (Z-test)	*P* value
AFP (IU/ml)	4099.0 ± 1381.0	60.0 ± 53.84	2.6 ± 0.42	59.125	<0.001*
KL-6 (U/ml)	621.43 ± 257.19	319.7 ± 117.82	236.52 ± 95.68	61.9	<0.001*

P value is highly significant at <0.001*.

Table (3): Spearman's correlation coefficient between AFP and KL-6 in all patients (N=103).

	r	Sig (2-tailed)	*P* value
AFP & KL-6	0.625	0.000	<0.001*

P value is highly significant correlation at <0.001*.

Table (4): Descriptive statistics of the studied parameters (AFP & KL-6) in HCC group and LC group.

	HCC group (N=57)				LC group (N=46)			
	+ ve		-ve		+ ve		- ve	
	No	%	No	%	No	%	No	%
AFP (IU/ml)	39	68.4	18	31.6	19	41.3	27	58.7
KL-6 (U/ml)	48	84.2	9	15.8	24	52.2	22	47.8

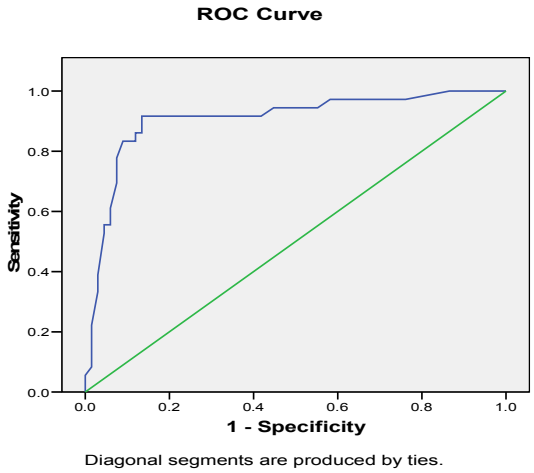
Table (5) KL-6 serum levels in relation to Child classification in all patients (No: 103 patients)

	No	%	Mean ± SD	Test of sign.	P value
Child A	28	27.2	323.5±149.15	31.3	<0.001*
Child B	24	23.3	1086.6±401.6		
Child C	51	49.5	3799.1±416.9		

P value is highly significant correlation at <0.001*.

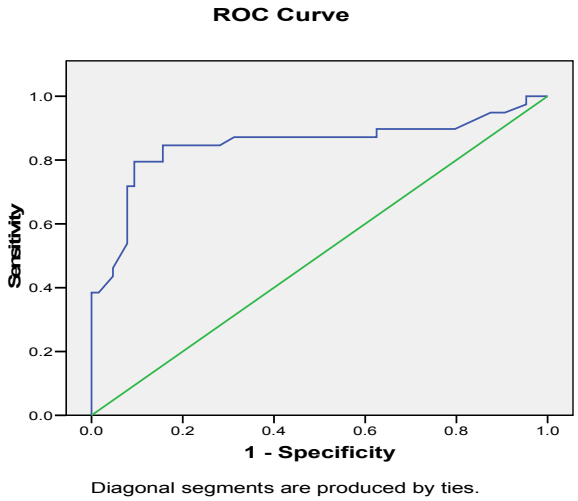
Table (6) KL-6 serum levels in relation to tumor size in HCC patients (No: 57 patients)

	No	%	Mean ± SD	Test of sign.	P value
< 3cm	15	26.3	424.5±211.9	74.3	<0.001*
3-5 cm	25	43.9	518.7±225.1		
> 5 cm	17	29.8	752.3±322.3		



Area under curve (AUC)	Standard error (SE)	95 % Confidence interval (CI)	
		Lower bound	Upper bound
0.906	0.034	0.840	0.973

Figure (1): ROC curve of KL-6 as a test variable in comparison with AFP in the patients (N= 103).



Area under curve (AUC)	Standard error (SE)	95 % Confidence interval (CI)	
		Lower bound	Upper bound
0.848	0.047	0.757	0.940

Figure (2): ROC curve of AFP as a test variable in comparison with KL-6 in the patients (N= 103).

Table (7): ROC curves analysis of the studied parameters (AFP & KL-6) in the patients (N=103).

	Sensitivity (%)	Specificity (%)	Cut-off point	PPV (%)	NPV (%)
AFP (IU/ml)	84.6	89.4	102	84.6	49.5
KL-6 (U/ml)	91.7	85.7	437	49.5	49.5

PPV: Positive predictive value NPV: Negative predictive value

4. Discussion

Hepatocellular carcinoma (HCC) is one of the commonest cancers worldwide, particularly in countries of the developing world and is increasing in incidence. The slow development and the late detection of HCC suggest that the identification of biomarkers of disease progression and early detection represents an attractive strategy for potential improvement of the outcome of HCC patients [24]. **Sherman, 2001** [25] stated that serum AFP is the most commonly used marker for HCC neoplasm, but its real clinical usefulness is unclear & furthermore, the role of AFP in HCC screening and diagnosis has lost most of the appeal that it had in the presophisticated (i.e. multislice, contrast-enhanced computed tomography, magnetic resonance imaging, etc.) imaging era. Because of the reported sensitivity (39% - 65%) and specificity (76% - 94%) of serum AFP are not sufficient for early diagnosis of HCC, additional effective markers are needed [7].

Aim of this study was to investigate the serum levels of KL-6 and AFP in HCC Egyptain patients, proving its role as a new diagnostic and prognostic marker of early detection of HCC in Egypt.

In the present study a statistically significant difference in the mean of the serum level of AFP between the three groups with highest increase in the HCC group and a slight increase in the cirrhotic group. These findings agreed with **Lau and Lai, 2008** [1] as they stated that the diagnosis of HCC is typically made by radiological liver imaging in combination with serum AFP but marginal elevations are common in patients with chronic hepatitis or cirrhosis.

Trerotoli and his colleagues, 2009 [9] stated that AFP, the only marker commonly used in clinical practice, displays poor sensitivity and a high specificity only for values higher than 400 IU/ml. Also **Farinati and his coworkers, 2006** [26] reported that because AFP concentrations are directly with tumor size, the reliability of such marker appears inadequate for early recognition of HCC.

Regarding the mean serum level of KL-6, a highly statistical significant difference was detected between HCC, LC and control groups with marked increase in the HCC group. **Gad and his coworkers, 2005** [27] agreed with these results as they found a significantly higher mean KL-6 in HCC compared

with non-HCC; either with or without LC; in addition no difference in mean KL-6 was found among HCC patients with and without LC. They stated that such findings together point to KL-6 association with HCC independent on the presence or absence of LC.

KL-6 serum levels were found to be significantly elevated with higher Child scores. This comes in accordance with **Suzuki *et al.*, in 2003** [28] found that levels of serum KL-6 of liver cirrhosis patients were significantly higher than that for the chronic hepatitis patients and when liver cirrhosis was classified according to Child's system, the level of serum KL-6 for the Child B/C patients was significantly higher than that for the Child A patients. Thus they suggested a correlation between KL-6 and liver disease, stating that this marker reflects hepatic fibrosis even better than pulmonary fibrosis.

The current study showed a highly statistical significant positive correlation between AFP and KL-6. **Gad *et al.*, 2005** [27] disagreed with these results as they stated that KL-6 serum level did not correlate with either serum AFP or PIVKA-II levels which points to its behavior independently from either of them and this may justify its clinical significance as an independent tumor marker for HCC diagnosis when considered with both AFP and PIVKA-II. This difference may be due to most patients of this study were HCV + ve as **Moriyama and his colleagues, 2003** [29] stated that there is a clinical significance of KL-6 as a tumor marker in HCV-related HCC.

The present study showed that 84.2% (48 patients) of HCC group having elevated serum level of KL-6, while 68.4% (39 patients) of the same group having elevated serum level of AFP. Regarding the cirrhotic group, 52.2% (24 patients) showed elevated serum level of KL-6 compared to 41.3% (19 patients) only showed elevated serum level of AFP in the same group. So, these data indicate that KL-6 could be a promising tumor marker for early detection for HCC.

Soresi *et al.*, 2003 [30] agreed with the above results as they stated that the false negative rate with AFP level alone may be as high as 40% for patients with early stage HCC and even in patients with advanced HCC, the AFP levels may remain normal in 15%-30% of the patients. Also, **Franca and his coworkers, 2004** [31] reported that up to 42% of patients with HCC present with serum AFP levels within normal values.

Another positive correlation in this study was found between serum levels of KL-6 and the tumor size revealing that this marker is related to the HCC tissue. this postulation does not correlate with the study done by **Gad *et al.*, 2005** [27], which revealed that no significant association with tumor size, echogenicity or multiplicity with significantly lower mean KL-6 was noticed in larger size tumors of >5 cm in comparison with tumors of less than or equal to 5 cm.

Applying the ROC curves analysis showed the best cut-off value of KL-6 to detect HCC was 437 U/ml with sensitivity 91.7% and specificity of 85.7% and the best cut-off value of AFP was 102 IU/ml with 84.6% and 89.4% in sensitivity and specificity respectively. **Gad and his coworkers, 2005** [27] used a cut-off point of 334 U/ml in his analysis of KL-6 as a tumor marker in patients with HCC. This cut-off gave the best sensitivity (60%) in their study.

Regarding the AFP cut-off level for the diagnosis of HCC**, Goma and his colleagues, 2003** [7] revealed an AFP value above 400-500 ng/ml has been considered to be diagnostic for HCC in patients with cirrhosis. Also, **Lau and Lai, 2008** [1] stated the specificity of AFP is very high when the levels are above 400 ng/ml in patients without testicular tumor. **Yao *et al.*, 2007** [32] reported that it is sometimes very difficult to make the distinction between tumors and falsely elevated AFP levels because of benign liver diseases.

In conclusion, this study suggests that KL-6 could be a good marker for detection of HCC in Egyptian patients. A large scale study is needed to investigate its clinical usefulness in screening cirrhotic patient for HCC.

Corresponding author:
Soha Zaki El-Shenawy.
Departments of [1]Clinical Biochemist, National Liver Institute, Menofiya
sohazaki69@yahoo.com.

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