The Egyptian Journal of Hospital Medicine (April 2019) Vol. 75 (6), Page 3027-3032

Kallistatin as a New and Reliable Biomarker for the Diagnosis of Liver Cirrhosis

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ABSTRACT

Background: Cirrhosis represents the final common pathological outcome for the majority of chronic liver diseases. Most patients with cirrhosis die from one or more clinical complications including ascites, hepatic encephalopathy and variceal hemorrhage.

Objective: The aim of the study was to explore the relationship between serum kallistatin and clinical evidence of cirrhosis and to determine if serum kallistatin levels could be used as a diagnostic indicator of hepatic health status especially human liver cirrhosis.

Patients and methods: The present study included 70 patients with liver cirrhosis and 25 healthy volunteers served as controls. Cirrhotic patients were subdivided into groups A, B and C according to Child score.

Results: The results in the current study revealed that there was highly significant decrease of kallistatin level in liver cirrhosis patients compared to control group. The level of kallistatin was significantly decreased in decompensated patients more than compensated patients. These findings pointed to the value of kallistatin as a new biomarker for diagnosis of liver cirrhosis.

Conclusion: Our findings support that kallistatin may be an efficient biomarker in early detection of liver cirrhosis. Also combination of kallistatin with Fib4 score could improve the sensitivity and specificity for chronic liver disease.

Keywords: Cirrhosis, Kallistatin, Encephalopathy.

INTRODUCTION

Liver cirrhosis (LC) is the final common pathological pathway of liver damage arising from a wide variety of chronic liver diseases **(1).**

Most patients with cirrhosis die from one or more clinical complications including ascites, hepatic encephalopathy and variceal hemorrhage **(2)**. Among the 1.4 million liver disease-related deaths that occur each year worldwide, over 55%, or 796,000, are directly attributable to cirrhosis **(3).**

Liver biopsy (LB) is the gold standard for appraising hepatic fibrosis. However, this procedure has several drawbacks including being invasive test with the risk of omplications, high cost, high rate of refusal by patients and sampling errors, which led to approximately 10–30% false negative result in cirrhotic patients **(4).**

It is important to identify reliable biomarkers for the early detection of liver disease and subsequent evaluation of response to therapeutic intervention as there is evidence of either fibrotic or cirrhotic regression has now been reported in chronic liver diseases of different etiologies, including viral hepatitis **(5).**

Kallistatin, an endogenous human serine proteinase inhibitor, was originally known as a tissue kallikrein inhibitor **(6).** Kallistatin has vasodilatory, anti-angiogenic, anti-inflammatory, anti-tumor and anti-oxidant effects **(7).** The significantly reduced levels of serum kallistatin in patients with LC hypothesized that serum kallistatin levels could be a potential biomarker for liver cirrhosis as several studies have shown that the liver represents the major site of synthesis and secretion of kallistatin **(8).**

AIM OF THE WORK

The aim of the study was to explore the relationship between serum kallistatin and clinical evidence of cirrhosis and to determine if serum kallistatin levels could be used as a diagnostic indicator of hepatic health status especially human liver cirrhosis.

MATERIAL AND METHODS

The present study included 75 patients with liver cirrhosis and 25 healthy volunteers served as controls. Cirrhotic patients were subdivided into groups A, B and C according to Child score. **The study was approved by the Ethics Board of Al-Azhar University and an informed written consent was taken from each participant in the study.**

Collection of blood samples:

Blood samples were collected from Damanhur Medical National Institute and serum was separated by centrifuging of clotted blood at 4000 rpm at 4 ^{o}C for 10 minutes and then stored at - 80 ^{o}C until testing of biochemical parameters. Additional blood samples were collected in tubes containing sodium citrate centrifuged at 4000 rpm for 10 minutes and then plasma was collected immediately for testing clotting parameters.

Received:12/2/2019
Accepted:14/3/2019

All patients were subjected to the following:

a. Alanine transaminase (ALT), aspartate transaminase (AST), albumin, total bilirubin, alpha feto protein (AFP), total protein, Kidney function tests: BUN, creatinine and CBC.
b. HCV antibodies using ELISA technique and HBsAg.
c. Special test: fibrosis 4 score "It is a non-invasive scoring system based on several laboratory tests that help to estimate the amount of scarring in the liver, the formula is: (age x AST)/ (platelets x sqr [ALT])".
d. Measurement of serum kallistatin using enzyme linked immunosorbent assay (ELISAusing affymetrix kits (Bioassay, Shanghai, China).
e. Abdominal ultrasonography scan for patient and controls.

Statistical Analysis

1) **One way ANOVA** test was used to compare more than two groups as regard quantitative variable.
2) **ROC-curve: Receiver Operating Characteristic curve analysis** is a graphical plot which illustrates the performance of a binary classifier system as its discrimination threshold is varied.
2. **Fisher's Exact or Monte Carlo correction:** Correction for chi-square when more than 20% of the cells have expected count less than 5.
3. **Student t-test:** For normally quantitative variables, to compare between two studied groups.
4. **Pearson coefficient:** To correlate between two normally quantitative variables.
5. **Mann Whitney test:** For abnormally quantitative variables, to compare between two studied groups.

RESULTS

This study was conducted on 70 patients and 25 control subjects recruited from Damnhour Hospital in the period from December 2017 to July 2018. They were divided into two groups:

- **Group I:** Included 70 liver cirrhosis patients, with 73% male patients and 27% female patients.
- **Group II:** Included 25 normal control, with 68% male individuals and 32% female subjects.

Further subdivision of cirrhotic patients according to child pugh score into 24 patient with child score A and 23 patient with child score B and 23 patient with Child score C.

Table (1): Comparison between the studied groups regarding characteristics of the studied patients

Variables		Control group		Liver cirrhosis		Statistic test	
		N=25		N=70		X^2	P
Sex	Male	17	68%	51	72.9%		
	Female	8	32%	19	27.1%	0.928^	0.503
Smoking	Negative	19	76%	46	65.7%		
	Positive	6	24%	24	34.3%	0.902[b]	0.342

^= montecarlotest [b]= chi square test * = significant ≤0.050crosstab

The previous table showed non-significant difference between control subjects and cirrhotic patients regarding sex distribution and smoking.

Table (2): Comparison between the studied groups regarding viral markers

		patient case		Total	Pearson Chi-Square
		liver cirrhosis n=70	Healthy individuals as control n=25		
HcvAb	**yes**	64	0	64	Pearson Chi-Square 28.056 P value 0.000*
		91.4%	0.0%	67.3%	
	no	6	25	31	
		8.6%	100.0%	32.7%	
Total		70	25	95	
		100.0%	100.0%	100.0%	

The previous table showed highly significant difference between control subjects and cirrhotic patients regarding hcv infection

Table (3): HBV sAg * patient case Cross tabulation

		patient case			Fisher's Exact Test
		liver cirrhosis n=70	healthy individuals as control n=25	**Total**	
HBV sAg	**yes**	6	0	6	Fisher's Exact Test =2.287 P=0.335
		8.6%	0.0%	6.3%	
	no	64	25	89	
		91.4%	100.0%	93.7%	
Total		70	25	95	
		100.0%	100.0%	100.0%	

The previous two table showed that there was no significant difference regarding hepatitis B infection**.**

Table (4): Comparison between the studied groups regarding age and laboratory data of the studied groups.

Variables		**Control group**	**Liver cirrhosis**	**Independent Samples Test**	
		N=25	**N=70**	**t**	**P**
Age (years)	Mean ± SD	56.32 ± 16.183	58.11 ± 9.095	0.300	0.765
Total bilirubin (µmol/L)	Mean ± SD	0.84 ± 0.12	2.92 ± 1.48	6.04	0.001*
Direct bilirubin (µmol/L)	Mean ± SD	0.19 ± 0.05	1.72 ± 1.14	5.311	0.001*
Albumin (g/L)	Mean ± SD	4.272 ± 0.27616	2.9971 ± 0.62	-15.323	0.001*
Aspartate Aminotransferase (U/L)	Mean ± SD	28.52 ± 5.606	42.76 ± 20.232	5.341	0.001*
Alanine aminotransferase (U/L)	Mean ± SD	23 ± 6.14	34 ± 16.61	4.711	0.001*
Creatinine (mg/dL)	Mean ± SD	0.97 ± 0.14	1.16 ± 0.25	4.737	0.102
BUN (mmol/L)	Mean ± SD	13.84 ± 3.555	16.87 ± 7.472	2.656	0.009*
Total protein (g/dL)	Mean ± SD	7.44 ± 0.11	6.92 ± 0.22	-0.592	0.085

The previous table showed that there was statistically significant difference between the control group and patients group regarding total and direct bilirubin, AST, ALT, Alb and BUN. While, there was no significant difference regarding serum creatinine and total protein. In addition, the two groups were nearly at the same age**.**

Table (5): Comparison between the cirrhotic patients regarding serum kallistatin

Variables		**Child score A**	**Child score B**	**Child score C**	**One way ANOVA tukey way**		
		N=24	**N=23**	**N=23**	**F**	**P**	**p**
Kallistatin	**Mean ± SD**	186.39 ± 26.87	146.04 ± 13.76	112.5 ± 31.87	7.933	0.001	P1=0.002* P2=0.000* P3=0.007*

The previous table showed that there was statistically significant difference found between the three groups regarding kallistatin level. The difference was by increase.

Table (6): Relation between kallistatin and clinical complication in liver cirrhosis group

Variables	Kallistatin Mean ± SD		Independent Samples Test	
	YES	NO	t	p
Ascites	99 ±36	132 ± 33	2.575	0.039*
Bleeding	125 ± 26	143 ± 34	1769	0.09
Encephalopathy	88 ± 19	147 ± 41	1.593	0.023*
Jaundice	101 ± 37	119 ± 38	1.026	0.032*

The previous table showed that there was statistically significant decrease in level of kallistatin in patients with ascites, encephalopathy and jaundice than those without.

Table (7): Correlation between kallistatin and other variables

Variables		
	r	p
Child Pugh score	-0.606*	0.001*
Total bilirubin (μmol/L)	-0.313	0.009*
Direct bilirubin (μmol/L)	-0.329	0.006*
Prothrombin time	-0.599	0.032*
Albumin (g/L)	0.305	0.012*
T. protein (g/dL)	-0.005	0.985
Aspartate Aminotransferase	0.030	0.854
Alanine aminotransferase (IU/L)	-0.076	0.641
International normalized ratio (INR)	-0. 573	0.023*
FIB4 scoring	-0.512	0.019*
BUN (mmol/L)	0.191	0.238
Creatinine (mg/dL)	0.009	0.956
Hemoglobin (g/dl)	0.374	0.051*
Blood platelets	0.432	0.004*
Alpha Fetoprotein	-0.452	0.008*

The previous table showed that there was statistically significant positive correlation between kallistatin and Alb, Hb and Plt. However, there was statistically significant negative correlation with total, direct bilirubin, PT, INR, Alpha Feto Protein, Child pugh and FIB4 scoring but no other significant correlation found between kallistatin and the other studied parameters.

Table (8): ROC curve of liver cirrhosis group and control group

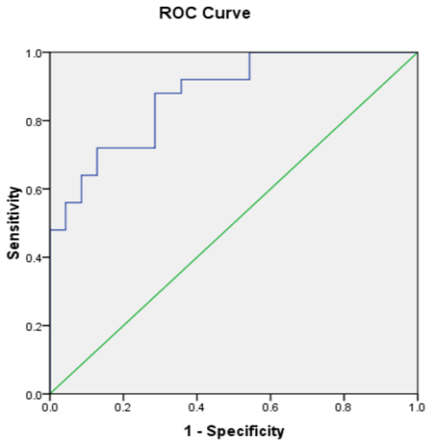
Area Under the Curve

Test Result Variable(s):KS

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.876	.039	.000	.800	.952

a. Under the nonparametric assumption

Cut off point	AUC	Sensitivity	Specificity	+PV	-PV
≤224.34	0.876	91.62	89	77.9	81.9



The previous ROC curve showed that the best cut off point for predicting liver cirrhosis patients from the control group as regard kallistatin was found to be ≤ 224.34 ng/mL with sensitivity of 91.62% and specificity of 89% and area under the curve of 0.876**.**

DISCUSSION

Cirrhosis is an important cause of morbidity and mortality world-wide and according to the WHO about 800,000 people die of cirrhosis annually. Because chronic liver disease affects people in their most productive years of life, it has a significant impact on the economy as a result of premature death, illness, and disability **(9).**

The prevalence of chronic liver disease/cirrhosis worldwide is estimated to be 100 (range 25 to 400) per 100,000 subjects, but it varies widely by country and by region **(9).**

The definitive diagnosis of cirrhosis relies on the histological examination of liver tissue. The use of liver biopsies in clinical practice, however, has several limitations. In particular, sample errors are a significant problem, with an estimated mean of 24% of false negatives being reported in series of blind liver biopsies **(10).**

Kallistatin, an endogenous human serine proteinase inhibitor, was originally known as a tissue kallikrein inhibitor **(6).** The significantly reduced levels of serum kallistatin in patients with LC hypothesized that serum kallistatin levels could be a potential biomarker for liver cirrhosis as several studies have shown that the liver represents the major site of synthesis and secretion of kallistatin **(8).**

In the current study, a statistically significant decrease in the mean of platelet counts was observed in patients with chronic hepatitis C (CHC) than control subjects. As expected, mean platelet count in the advanced cirrhosis group was extremely decreased compared to those in early cirrhosis group and the control subjects and these suggested that platelet count might be helpful in the assessment of chronic liver diseases. CHC has been reported as one of the several causes that induce thrombocytopenia, even in chronic non-cirrhotic patients **(11).** Thrombocytopenia in chronic liver disease may be explained by suppression of platelet production by the bone marrow as a result of viral infection, alcohol consumption, iron overload, and medications. Splenic sequestration of platelets due to hypersplenism may be another cause of the reduction in the platelet numbers **(12).**

Regarding serum kallistatin levels, the present study revealed highly significant lower serum levels of kallistatin among patients with liver cirrhosis as compared to control group with cutoff point of ≤ 224 ng /mL, sensitivity of 91.62% and specificity of 89%. This result comes in accordance with the study of **Cheng and coworkers (10)** where they reported significant lower serum kallistatin levels in patients with liver cirrhosis as the liver represents the major site of synthesis and secretion of kallistatin**.**

Elsaeed *et al.* (13) had other predictive values as they found that, kallistatin serum level ≥ 40.6 ng⁄ml (cutoff) diagnose compensated cirrhosis by 93.33% sensitivity, 86.67% specificity, 87.50% positive predictive value and 92.85% negative predictive value.

This study revealed negative correlation between kallistatin and both Child score and fibrosis 4 score suggesting that serum levels of kallistatin may reflect the degree of liver dysfunction. This study comes in line with the study of **Yin *et al.* (14)** who stated that kallistatin levels in patients with compensated liver disease were higher than patients with decompensated liver disease.

This study also demonstrated that the level of serum kallistatin was reduced significantly in patients with liver cirrhosis. The magnitude of this decrease appeared to be correlated with the degree of liver cirrhosis and disruption of normal liver function. This study is in agreement with the results of **Cheng *et al.* (10)** who reported that Kallistatin considered a potential new biomarker for the diagnosis and evaluation of the extent of human liver cirrhosis. Furthermore, kallistatin may provide a new therapeutic strategy for the management and treatment of cirrhotic liver damage.

CONCLUSION

Our findings support that kallistatin may be an efficient biomarker in early detection of liver cirrhosis. Also combination of kallistatin with Fib 4 score could improve the sensitivity and specificity for chronic liver disease.

RECOMMENDATIONS

Our study was limited because of the small sample size, we recommend further studies with larger number of participants.

Most participants in this study were from the Egyptian population and we examined chronic liver disease induced by HCV-infection with no information if our findings would be valid for other patients with different etiologies. Thus, further investigation should be carried out in order to validate these findings.

Participants in this study with chronic liver disease induced by HCV-infection did not have the antiviral treatment. Thus, we recommend further studies to detect serum kallistatin before and after treatment.

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