Serum kallistatin as a marker of severity of liver fibrosis in cirrhosis: A cross-sectional observational study

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ABSTRACT

Background: Liver cirrhosis is among the leading causes of morbidity and mortality worldwide. Although liver biopsy is the gold standard for the assessment of liver fibrosis in cirrhosis, it has its own limitations. Therefore, noninvasive methods to detect liver fibrosis are widely preferred. However, they also have their own limitations. Thus, there is always a need to extend the battery of serum-based assays. Kallistatin is a protein synthesized primarily in the liver. As it is a negative acute-phase protein, its blood level decreases with a decline in liver function. In our study, we explored the relationship between serum kallistatin and radiological evidence of liver fibrosis by transient elastography to determine if kallistatin levels can be used as a diagnostic marker of liver fibrosis.

Materials and Methods: A cross-sectional study of 1-year duration was conducted at a leading tertiary care hospital in northern India. Patients between 15 and 75 years of age having evidence of chronic liver disease were enrolled. All enrolled patients were evaluated by detailed history, physical examination, and relevant investigations. Serum kallistatin levels were quantified using the ELISA method. Grading of liver fibrosis was done using transient elastography. A FibroScan scoring card was used to convert FibroScan results measured in kPa into the Metavir scale F1–F4. Results: A total of 128 subjects, including 64 patients with cirrhosis and 64 healthy controls, were enrolled. Our study suggested that FibroScan values were significantly higher in cases as compared to controls. The kallistatin level of cases was significantly lower than that of controls. An inverse correlation was found between FibroScan value and kallistatin level among cases. Conclusion: We conclude that serum kallistatin levels are low in patients with liver fibrosis and can be used as a potential marker of liver fibrosis.

Keywords: Cirrhosis of liver, kallistatin, transient elastography

Introduction

Liver cirrhosis (LC), a final outcome of all chronic liver diseases, is a pathologic entity characterized by diffuse hepatic fibrosis with the replacement of the normal liver architecture by regenerating nodules.[3] Diagnosis of patients of LC in a resource-poor setting is often delayed because of the nonavailability of liver biopsy and elastography. Most of such patients present with life-threatening complications, contributing to significant morbidity and mortality.[2]

Liver biopsy is the gold standard for the assessment of hepatic fibrosis in liver cirrhosis.[3] However, it is not widely accepted as it is an invasive method, with a risk of bleeding and a reported...
mortality rate of 1 in 10,000. Moreover, there is significant inter-observer variability in the interpretation of liver biopsy findings and the possibility of sampling error varying between 33% and 50%. Due to these limitations, researchers are trying to replace liver biopsies with noninvasive methods such as serum biomarkers.

Noninvasive methods to estimate hepatic fibrosis are preferred in clinical practice as they are comparatively safer, more accessible, and cheaper than a liver biopsy. These methods include indirect biomarkers, direct biomarkers, and elastography. Some serum markers have been found better than biopsy in excluding advanced fibrosis from mild-fibrosis patients. Nowadays, the optimal approach to liver fibrosis assessment is to use noninvasive serum markers in conjunction with transient elastography. If transient elastography is not available, two different noninvasive serum markers/tests can be used.

Currently available direct markers of fibrosis are procollagen (type I, III, and IV), matrix metalloproteinases, cytokines, and chemokines. Hyaluronic acid is a glycosaminoglycan secreted by hepatic stellate cells. Extensive fibrosis/cirrhosis has been found to be associated with increased serum levels of hyaluronic acid. The direct markers have shown variable effectiveness in predicting liver fibrosis. The currently available direct markers lack sensitivity in identifying patients with a mild degree of fibrosis. Moreover, these markers are often not specific because they can be detected in organs other than the liver and can be affected by other pathological conditions, such as renal or liver failure.

Considering the drawbacks of liver biopsy and biomarkers (direct and indirect), there is always a need to extend the battery of serum markers that can enhance the sensitivity and accuracy of currently employed biomarkers tests.

Kallistatin is a protein synthesized and secreted primarily in the liver. It is also secreted in low concentration from eyes, kidneys, liver, pancreas heart, arteries and veins, atheroma, blood cells, and body fluids. It functions as a vasodilator, anti-inflammatory, anti-oxidant, anti-angiogenic, and anti-tumor growth protein. As it is a negative acute-phase protein, its blood level decreases with a decline in liver function. Thus, serum kallistatin levels can be a potential biomarker for liver cirrhosis and fibrosis. Transient elastography, marketed as FibroScan, is a widely acceptable radiological test to assess the grade of liver fibrosis in patients with liver cirrhosis.

It is always challenging to assess the severity of liver fibrosis for primary care physicians working in a resource-constrained setting, where facilities of liver biopsy and FibroScan are usually not available. Our study explored the relationship between serum kallistatin and radiological evidence of liver fibrosis assessed by transient elastography. This study will enable physicians to assess the severity of liver fibrosis by using serum kallistatin so that appropriate steps can be taken timely for better outcomes for patients.

### Materials and Methods

Our study was a cross-sectional observational study of 1-year duration conducted in the Department of Medicine, King George's Medical University, Lucknow, a leading tertiary care hospital of India. The patients with cirrhosis of liver admitted in the Department of Medicine, KGMU, Lucknow who fulfilled the inclusion criteria were included in the study.

Ethical approval was taken before the initiation of the study from the institutional ethical committee of King George's Medical University, Lucknow (U.P), India (Letter number-Ref. code 84th ECM/B-Thesis/P31).

Patients presenting with symptoms of hepatocellular dysfunction (jaundice, gastrointestinal bleed, edema, ascites, gynecomastia), presence of portal hypertension as evidenced by ultrasonographic findings of portal vein diameter more than 13 mm, splenomegaly, and esophageal and/or gastric varices on gastro-duodenoscopic examination were identified as cirrhosis of the liver.

Because most of the cirrhotic patients admitted in our indoor wards were chronic alcoholic and chronic hepatitis B Band C, we preferentially enrolled them if they fulfilled the following inclusion criteria.

#### Inclusion criteria

1. Patients of age 15–75 years
2. HCV RNA positive along with disease evidence greater than 6 months of duration
3. Hepatitis B infection with continued evidence of inflammation and necrosis for a time period of 6 months or greater along with hepatitis B surface antigen-positive for > 6 months
4. Alcohol intake of >60–80 g per day for 10 years in men and >20 g per day for 10 years in women

Patients with cardiovascular disease, chronic kidney disease, pancreatic disease, and diabetes mellitus were excluded from the study to avoid false-positive results. We also enrolled 64 healthy controls in our study so that we could compare kallistatin levels of cirrhotic patients with healthy controls. All the healthy controls had normal ultrasound abdomen and liver function tests.

All subjects enrolled in the study were evaluated by detailed history regarding chronic liver disease and physical examination. Grading of fibrosis was done using transient elastography (FibroScan). FibroScan scoring card is used to convert FibroScan results (measured in kPa) into the Metavir scale F1–F4.

After 8 h of fasting, a peripheral venous blood sample was obtained. Serum kallistatin levels were quantified by ELISA (QAYEE-BIO for life science) kit at Central Drug Research Institute, Lucknow, a leading research institute in north India.
Statistical analysis

Data were summarized as Mean ± SE (standard error of the mean). Groups were compared by independent student’s t test. Groups were also compared by one-factor analysis of variance (ANOVA), and the significance of the mean difference between the groups was done using Newman–Keuls post hoc test after ascertaining normality by Shapiro–Wilk’s test and homogeneity of variance between groups by Levene’s test.

Categorical (discrete) groups were compared using the Chi-square (χ²) test. Pearson correlation analysis was done to assess the association between the variables. A two-tailed (α = 2) P < 0.05 was considered statistically significant. Analyses were performed on SPSS software (Windows version 17.0).

Results and Observations

Our study correlated liver fibrosis grading assessed by transient elastography and serum kallistatin levels in chronic hepatitis B and C and alcoholic liver disease. In total, 128 subjects—64 with liver disease (cases) and 64 normal healthy subjects without liver disease (controls)—were enrolled. Out of the 64 cases, 22 were chronic hepatitis B, 15 were chronic Hepatitis C, and 27 were patients with alcoholic liver disease.

The results of our study are summarized below:

1. Demographic characteristics of cases and controls.
   
   The age and sex profile of enrolled patients are shown in [Table 1]. It shows that the age of controls and cases ranged from 19 to 85 years and 14 to 88 years, respectively, with a mean ± SE of 45.20 ± 3.22 and 44.72 ± 1.69 years, respectively (P = 0.892). Further, in controls, there were 26 (40.62%) females and 38 (59.37%) males, whereas in cases, 21 (32.8%) and 43 (67.2%) were females and males, respectively (P = 0.555). Thus, both controls and cases were comparable in terms of age and sex.

2. Comparison of FibroScan value of cases and controls
   
   The results of FibroScan value of cases and controls are summarized in [Table 2]. It shows that FibroScan value were significantly higher in cases (38.13 ± 2.54 kPa) as compared to controls (655.40 ± 143.49 kPa) (P < 0.001). There was no effect of etiology, age, or sex on FibroScan value.

3. Comparison of serum Kallistatin level of cases and controls
   
   The results of serum kallistatin levels of cases and controls are summarized in [Table 3]. It showed that serum kallistatin level of cases were significantly lower (171.60 ± 18.91 ng/mL) than controls (655.40 ± 143.49 ng/mL) (P < 0.001). The kallistatin levels did not appear to be influenced by etiology and age.

4. Correlation between FibroScan value and kallistatin level in cases (n = 64).
   
   The correlation between FibroScan value and kallistatin level among cases is summarized in [Table 4 and Figure 1]. It showed that among the cases, an inverse correlation was found between FibroScan value and serum kallistatin level, which was statistically nonsignificant (r = −0.15, P > 0.05).

Discussion

Liver cirrhosis (LC) is one of the most common causes of morbidity and mortality all over the world.[18] For primary care physicians working in a resource-constrained setting, diagnosis and grading of liver fibrosis is often delayed because of the nonavailability of liver biopsy and FibroScan. Majority of such patients with liver cirrhosis present with life-threatening complications. Thus, early diagnosis and treatment of LC is the key step to reduce mortality in patients with chronic liver disease.[19]

Our study showed that serum kallistatin levels in patients with liver fibrosis were significantly lower than those in healthy controls, demonstrating a correlation between the reduction in serum kallistatin levels and the severity of liver disease. This finding was similar to previous studies done by Sobhey et al.,[20] Elsaed et al.,[21] Cheng et al.,[22] and Chao et al.[23] Therefore, serum kallistatin levels can provide an additional biomarker for the detection of progressive loss of liver function leading to liver fibrosis and portal hypertension.

In our study, we also observed that the absolute value of serum kallistatin levels in cases was significantly lower than in previous

| Table 1: Age and sex profile of cases and controls |
|-----------------------------------------------|
| Demographic characteristics | Controls (n=64) (%) | Cases (n=64) (%) | χ²/α | P |
| Age (yrs) | 45.20±3.22 | 44.72±1.69 | 0.14 | 0.892 |
| Sex | | | | |
| Female | 26 (40.62) | 21 (32.8) | 0.35 | 0.555 |
| Male | 38 (59.37) | 43 (67.2) | | |

| Table 2: Fibroscan value (Mean±SE) of cases and controls |
|-------------------------------------------------------------|
| Controls (n=64) | Cases (n=64) | Mean diff (95% CI) | P |
| Mean FibroScan value (kPa) | 4.76±1.20 | 38.13±2.54 | 33.37±4.57 | <0.001 |
| (3.60–6.80) | (5.60–7.50) | (24.28–42.47) | |

| Table 3: Kallistatin level (Mean±SE) among cases and controls |
|-----------------------------------------------|
| Controls (n=64) | Cases (n=64) | Mean difference (95% CI) | P |
| Mean kallistatin level (ng/mL) | 655.40±143.49 | 171.60±18.91 | 483.80±86.11 | <0.001 |
| (12.79–2000.00) | (29.50–876.22) | (312.20–655.40) | |

| Table 4: Correlation between fibroscan value and kallistatin level in controls (n=64) and cases (n=64) |
|-----------------------------------------------|
| Group | Correlation (r) |
| Controls (n=64) | 0.41* |
| Cases (n=64) | −0.17* |

ns: P>0.05
In a resource-constrained setting, most cirrhotic patients are diagnosed late because of the nonavailability of liver biopsy and elastography.

Early diagnosis and appropriate treatment may improve clinical outcomes in such patients.

Serum kallistatin may be a promising tool to assess the severity of liver fibrosis in patients with liver cirrhosis.

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Nil.

Conflicts of interest

There are no conflicts of interest.

Conclusion

Our study concluded that there is a steady decline in serum kallistatin level with an increase in severity of liver fibrosis. In a resource-constrained setting, where facilities of liver biopsy and FibroScan are not available, serum kallistatin can be used as a novel marker of liver fibrosis. This will enable primary care physicians to take appropriate management steps which may improve the outcome of cirrhotic patients.

Key points

- Liver cirrhosis is a common cause of morbidity and mortality worldwide.
Novel role of kallistatin in protection against myocardial ischemia-reperfusion injury by preventing apoptosis and inflammation. Hum Gene Ther 2006;17:1201-13.

15.  Gao L, Yin H, Smith RS Jr, Chao L, Chao J. Role of kallistatin in prevention of cardiac remodeling after chronic myocardial infarction. Lab Invest 2008;88:1157-66.

16.  Miao RQ, Agata J, Chao L, Chao J. Kallistatin is a new inhibitor of angiogenesis and tumor growth. Blood 2002;100:3245-52.

17.  Wang CR, Chen SY, Wu CL, Liu MF, Jin YT, Chao L. Prophylactic adenovirus-mediated human kallistatin gene therapy suppresses rat arthritis by inhibiting angiogenesis and inflammation. Arthritis Rheum 2005;52:1319-24.

18.  Tsochatzis EA, Bosch J, Burroughs AK. Liver cirrhosis. Lancet 2014;383:1749-61.

19.  Benvegnù L, Gios M, Boccati S, Alberti A. Natural history of compensated viral cirrhosis: A prospective study on the incidence and hierarchy of major complications. Gut 2004;53:744-9.

20.  Sobhey OM, Jouda AA, Metwally A, Shawky NM, Elkhashab MN. Evaluation of serum kallistatin level as a predictor of esophageal varices in cirrhotic patients. Alex J Med 2020;36:21-6.

21.  Elsaeed AM, Ismail SM, Elgendy NA. Serum kallistatin and cholinesterase as biomarkers for the diagnosis of liver cirrhosis in patients with hepatitis C viral infection. Clin Med Diagn 2016;6:143-52.

22.  Cheng Z, Lv Y, Pang S, Bai R, Wang M, Lin S, et al. Kallistatin, a new and reliable biomarker for the diagnosis of liver cirrhosis. Acta Pharm Sinica B 2015;5:194-200.

23.  Chao J, Schmaier A, Chen LM, Yang Z, Chao L. Kallistatin, a novel human tissue kallikrein inhibitor: Levels in body fluids, blood cells, and tissues in health and disease. J Lab Clin Med 1996;127:612-20.