



Full Length Research Paper

Serum alanine aminotransferase (ALT) and aldosterone levels correlate with the severity of liver fibrosis in chronic viral hepatitis C

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Abstract

Background: In patients suffering from chronic hepatitis C (CHC), liver biopsy is performed to assess the severity of liver fibrosis. However, liver biopsy essentially is an invasive technique fraught with complications and its accuracy is also debatable due to sampling errors and subjective bias in the interpretation of the findings. **Aim:** To determine the correlation between the index of fibrosis in chronic hepatitis C and the serum level of various markers of liver damage and dysfunction. The serum markers analyzed were of two types (i) liver enzymes; alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyltranspeptidase (GGT); (ii) Serum markers of liver biosynthetic function: -albumin, prothrombin time (PT), high density lipoprotein (HDL), low density lipoprotein (LDL), haptoglobin and aldosterone. **Methods:** This was a case series study conducted over a 20-month period. The above mentioned serum markers in CHC patients who underwent ultrasonography (USG)-guided liver biopsy were evaluated. The biopsy specimen were examined by a single blinded investigator and was classified according to the degree of fibrosis into: mild fibrosis (MF) or severe fibrosis (SF) based on the Ishak score. **Results:** Among the 60 patients included in the study, 48 were classified as mild fibrotic (MF) while 12 were grouped as severe fibrotic (SF). Among the liver enzymes analyzed, the serum level of ALT ($p < 0.005$) and GGT ($p < 0.005$) were significantly higher in SF group while aldosterone ($p < 0.005$) was the only marker from the liver function group whose level was significantly higher in SF. On the other hand, among the markers of liver biosynthetic function, serum level of albumin ($p < 0.005$), HDL ($p < 0.005$) and haptoglobin ($p < 0.005$) were significantly higher in the MF group. Based on the comparative statistical analysis of the characteristics (Table II), only the higher ALT ($125 \text{ IU/L} \pm 31$; $p < 0.001$) and aldosterone ($184 \text{ pmol/L} \pm 43$; $p < 0.001$) levels were found to be predictors of severe fibrosis. **Conclusion:** Serum ALT and aldosterone levels correlate significantly with the severity of liver fibrosis. No significant correlation between the severity of liver fibrosis and the serum levels of AST, GGT, albumin, PT, HDL and haptoglobin was found in this study. Serum ALT and aldosterone levels may be used to monitor the progression of liver fibrosis.

Keywords: Chronic hepatitis C, liver fibrosis, alanine aminotransferase, aldosterone, biochemical markers.

INTRODUCTION

Liver biopsy is an imperfect gold standard for assessing the severity of fibrosis in chronic hepatitis C (CHC). Its limitations include sampling errors, the inter-observer/intra-observer variability in the interpretation of findings of histopathological specimens and the complications associated with the invasive nature of the procedure itself (Regev A et al, 2002; Skripenova S et al, 2006; Poynard T et al, 2000). Despite all these limitations, liver biopsy is still regarded as the standard technique for the assessment of progression of fibrosis and cirrhosis. For patients who have not received any treatment because of the mild nature of liver damage at the initial biopsy, a repeat liver biopsy at the intervals of 4–5 years is recommended (EASL, 1993).

It has been known for some time that patients with cirrhosis have elevated aldosterone levels (Coppage WS et al, 1962). Moreover, aldosterone antagonists like spironolactone are a mainstay in the management of ascites and fluid retention in cirrhosis (Runyon BA et al, 2004). The concept that renin-angiotensin-aldosterone system (RAAS) plays a role in vascular, myocardial and glomerular remodeling is relatively new (Duprez DA et al, 2006). Based on the findings of animal studies, a role for RAAS in hepatic fibrosis; and a role for the inhibitors of RAAS in arresting the progression of hepatic fibrosis has been postulated (Yoshiji H et al, 2006, Rombouts K et al, 2001, Töx U et al, 2006). These findings highlight the significance of hormones of RAAS as the biomarkers of liver fibrosis.

Design and development of non-invasive methods to identify the extent of fibrosis in CHC is an ongoing effort (Imbert-Bismut F et al, 2001). In this study, we investigated the role of serum aldosterone, aspartate aminotransferase (AST), alanine amino transferase (ALT), albumin, haptoglobin, prothrombin time (PT), low density lipoprotein (LDL) and high density lipoprotein (HDL) in the identification and characterization of liver fibrosis through a non-invasive method.

MATERIALS AND METHODS

Study population: The subjects of this study were selected from the following three tertiary care medical centers in northern Malaysia; Hospital Kulim, Hospital Sultan Abdul Halim, Sungai Petani and Hospital Sultanah Bahiyah, Alor Setar. Ethnically, the study population comprised 40 Malays, 17 Chinese and 3 Indians. Only patients who were 18 years old or above and were diagnosed with chronic hepatitis C infection were recruited for the study.

Inclusion criteria: All patients who underwent liver biopsy to determine the severity of hepatic fibrosis and were positive for hepatitis C by at least the second generation ELISA, and HCV RNA by polymerase chain reaction (PCR) were enrolled in the study. Ethics and

consent: Prior informed consent was obtained from all patients before engaging them in the study.

Exclusion criteria:

Patients with chronic renal failure. For the purpose of this study, chronic renal failure was defined as glomerular filtration rate (GFR) less than 60 mL/min/1.73m for more than 3 months. GFR was calculated using the Cockcroft-Gault formula (Cockcroft DW et al, 1976). -Patients with heart failure. For the purpose of the study, heart failure was defined as heart failure grade B or above as per the American College of Cardiology/American Heart Association 2005 guideline update for the diagnosis and management of chronic heart failure in the adult (Hunt SA, 2005). -Patients who were HIV positive and patients with other concomitant viral hepatitis. -Patients with diabetes mellitus and other co-morbid illness. -Patients who were on immunosuppressive drugs. -Patient with inadequate or uninterpretable liver biopsy sample.

Study design

Liver biopsy on all 60 patients in the study was performed under the guidance of ultrasound monitoring. Liver biopsy specimens, at least 10mm in length, were fixed, paraffin-embedded, and stained with hematoxylin-eosin, safranin and Masson's trichrome or picosirius red for collagen. Degree of fibrosis was graded on a scale of 0 to 6, based on the modified Ishak staging criteria (Ishak K et al, 1995; Bedossa P et al, 1996). The calculation of grading was done as follows: S0= no fibrosis, S1= fibrous expansion of some portal tracts ± short fibrous septa, S2= fibrous expansion of most portal areas ± short fibrous septa, S3= fibrous expansion of most portal areas with occasional portal to portal (PP) bridging, S4= fibrous expansion of portal areas with marked bridging [PP as well as portal-central (PC)], S5= marked bridging (PP and/or PC) with occasional nodules (incomplete cirrhosis), S6= cirrhosis, probable or definite.

All the blood tests except the tests for serum aldosterone and serum haptoglobin were performed in the laboratory of Hospital Alor Setar. The serum aldosterone and serum haptoglobin tests were performed using standard methods at Hospital Universiti Kebangsaan Malaysia and Gribbles laboratories, respectively.

The study populations were divided into two groups based on the severity of fibrosis; In group 1 patients who had mild fibrosis (MF) were graded from S0 to S3; Group 2: comprised of patients who had severe fibrosis and were graded from S4 to S6. The relationship between the serum level of hemoglobin, platelet, HDL, LDL, GGT, albumin, prothrombin time, AST, ALT, haptoglobin and serum aldosterone and the degree of fibrosis in liver were calculated using statistical analysis.

Table 1. Stages of fibrosis according to the Ishak scoring system.

Stages	n[total=60] (%)	Groups
S0	1(1.7%)	Group-1 Mild fibrosis 48(80%)
S1	40(66.7%)	
S2	1(1.7%)	
S3	6(10%)	
S4	8(13.3%)	Group-2 Severe fibrosis 12(20%)
S5	3(5%)	
S6	1(1.7%)	

Table 2. Comparison of study characteristics between mild (group-1) and severe fibrosis (group-2).

	Variable (units)	Group-1 mean \pm SD	Group-2 mean \pm SD	p-value
1	Haemoglobin (g/dL)	11.3 \pm 2.2	12 \pm 2	0.311
2	Platelet ($\times 10^3/\mu\text{l}$)	219.6 \pm 76	184 \pm 54	0.138
3	Albumin (g/L)	42 \pm 4	35 \pm 3.2	<0.001
4	Alanine transaminase (IU/L)	82 \pm 23	125 \pm 31	<0.001
5	Gamma-glutamyltranspeptidase (U/L)	47.5 \pm 31	81 \pm 25	<0.005
6	Prothrombin time (Seconds)	11.3 \pm 0.8	12 \pm 1.0	0.075
7	Aspartate transaminase (IU/L)	70 \pm 44.8	53 \pm 30	0.23
8	High density lipoprotein (mmol/L)	1.4 \pm 0.3	0.9 \pm 0.2	<0.001
9	Low density lipoprotein (mmol/L)	2.8 \pm 0.9	3 \pm 0.5	0.604
10	Haptoglobin (g/L)	0.7 \pm 0.6	0.3 \pm 0.2	0.11
11	Aldosterone (pmol/L)	111.6 \pm 56	184 \pm 43	<0.001

Statistical analysis

The particulars of patients as well as clinical and laboratory data of each patient included in the study were obtained from the medical records. These data were analyzed as frequencies while special emphasis was given to selected parameters that were known to influence the outcome of liver fibrosis based on the modified Ishak score. These parameters were patient age, gender, race and the serum levels of albumin, hemoglobin, AST, ALT, GGT, haptoglobin, serum aldosterone, LDL, HDL and prothrombin time. Normally distributed data were presented as mean and standard deviation whereas data not normally distributed were presented in median and interquartile range.

Statistical analysis was carried out using SPSS software program. To evaluate the relationship between serum level of HDL, LDL, hemoglobin, platelet, prothrombin time, AST, ALT, haptoglobin, Gamma-GT and serum aldosterone and the degree of liver fibrosis, compared mean followed by independent t-test was utilized. The accuracy of the results as mentioned above, for predicting significant fibrosis was also assessed by the area under the receiver-operating characteristic (ROC) curve. A p-value of 0.05 and less was taken as potentially significant association.

RESULTS

Reassignment of the study population based on the severity of liver fibrosis: The distribution of severity of fibrosis according to the Ishak scoring system among the 60 study population is shown in Table I. These patients were further divided into two groups: Group-1 representing the stages S0-S3 and comprising of patients with mild liver fibrosis; and group-2 represented the stages S4-S6 and comprised of patients with severe liver fibrosis. There were 48 (80%) patients in group-1 and 12 (20%) in group-2. The mean age of patients in group-1 was 41 \pm 11.6 years while for group-2 it was 50 \pm 8 years. Majority of patients in both groups were male; 36 (75%) in group-1 and 8 (66.7%) in group-2. The study characteristics of the two groups are compared in Table II.

Serum levels of selected liver enzymes in mild and severe liver fibrosis: Serum ALT level was markedly elevated in patients of group 2 (severe fibrosis) when compared to group 1 patients, the accuracy at 0.889 with CI 95% (0.82, 0.977) (Figure 1a). Serum Gamma-GT level was also significantly elevated in patients of group 2 (severe fibrosis) and the ROC curves showed the accuracy of GGT in predicting severe fibrosis at 0.788 (CI 95% 0.669, 0.908) (Figure 1b). Serum AST level showed

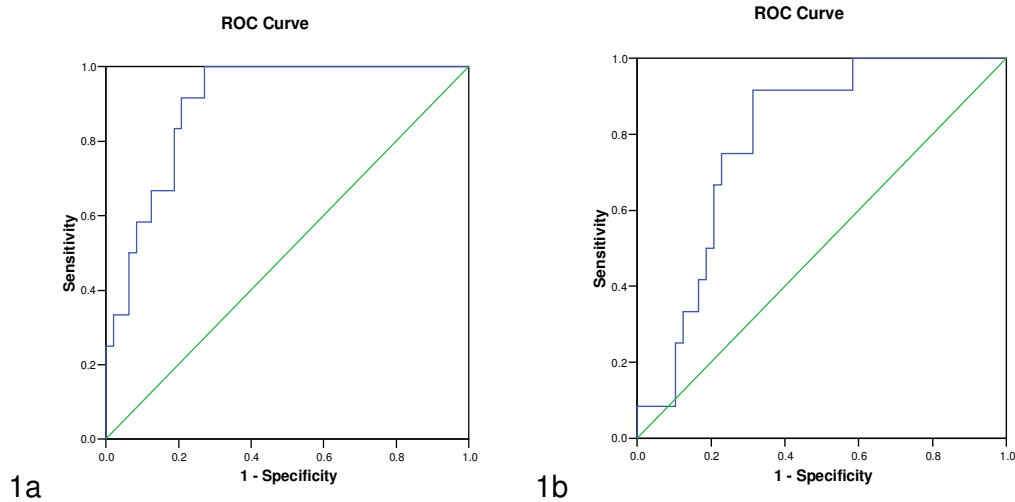


Figure 1a & 1b. Area under the operating characteristics curves (ROC) for alanine transaminase and gamma-GT for predicting severe fibrosis.

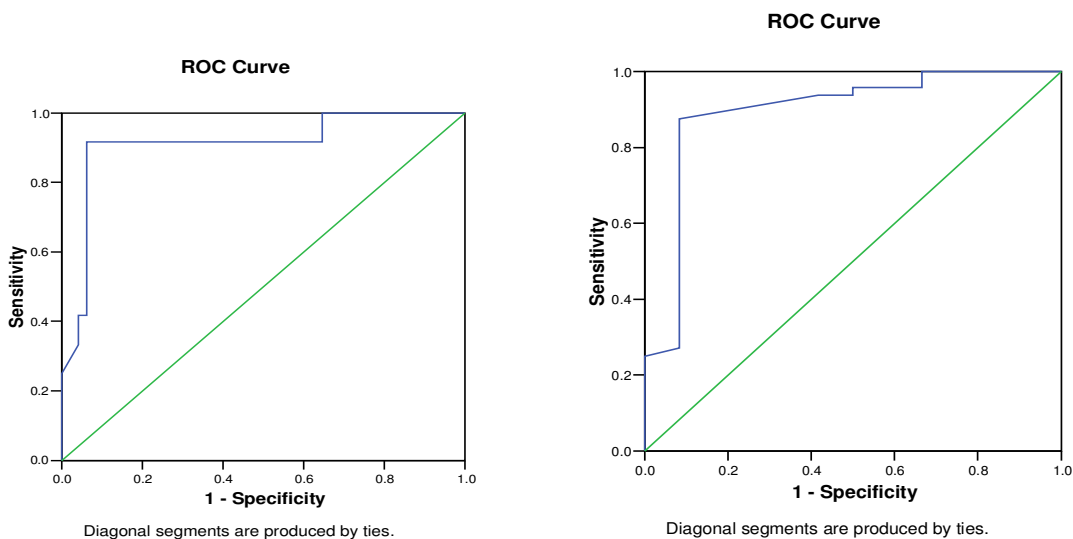


Figure 2a & 2b. Area under the operating characteristics curves (ROC) for low albumin for predicting severe fibrosis and low HDL for predicting mild fibrosis.

no positive correlation to the degree of liver fibrosis in patients of group 1 or 2.

Synthetic liver function and stages of liver fibrosis:

Serum levels of albumin and HDL were significantly lower in group 1 compared to group 2. Levels of serum albumin showed a positive correlation to mild stage of liver fibrosis as its level was significantly lower in patients of group 1 ($p < 0.001$). It has the area under the receiver operating characteristics curve (AUROC) at 0.9 CI 95% 0.80, 1.00. The synthetic liver function also showed positive correlation to the degree of liver fibrosis as serum level of high density lipoprotein (HDL) was significantly lower in group 1 patients ($p < 0.001$). As shown in the AUROC curves (AUROC), the level of HDL

has an accuracy of 10.5 % which is significantly low. The serum HDL level is usually reduced in more advanced stages of liver fibrosis, therefore the low AUROC of HDL level in our study may have resulted from a small percentage of study population in group 2; which was only 20% of total number of patients. This assumption is also supported by the larger upper bound value in ROC curves (95% CI – 0.14, 0.224). The serum HDL level has a good predictive value for mild fibrosis, with the AUROC being 0.85 (95% CI 0.776, 1.014). The accuracy of the test for low serum albumin was 91% with CI 95% (0.803, 1.010) (Figure 2a) and for low HDL was 89% at CI 95% (0.776, 1.01) (Figure 2b). It has a high accuracy rate in the mild stage of liver fibrosis. The LDL was not a good

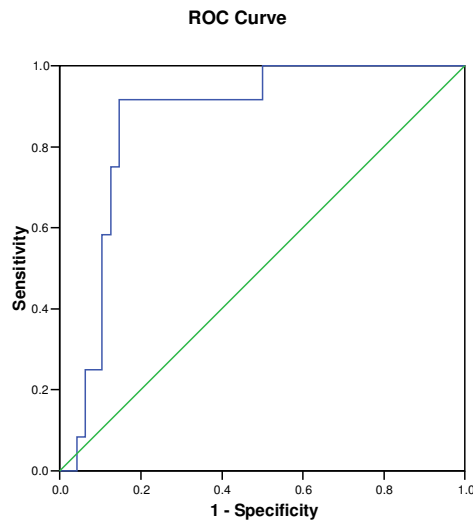


Figure 3. Area under the operating characteristics curve (ROC) for serum aldosterone for predicting severe fibrosis.

indicator for progression of liver fibrosis as shown in this study (p-value is 0.604). The accuracy of LDL was not significant as shown by the AUROC curve, at 0.57 (CI 95% 0.49, 0.741) for mild fibrosis and an AUROC curve at 0.425 (CI 95% 0.259, 0.591). The other synthetic liver functions, viz. prothrombin time, low density lipoprotein (LDL) and haptoglobin did not show a significant positive correlation with the degree of liver fibrosis.

Aldosterone and stages of liver fibrosis: The study showed a significant positive correlation of serum aldosterone level with the stages of liver fibrosis. The area under the receiver operating characteristics at CI 95% was 0.865 (0.764, 0.965) (Figure 3). The study demonstrates that the higher the serum aldosterone level the greater the chances of developing liver fibrosis as shown by statistical analysis. The AUROC showed the accuracy of this marker in relation to group 2 patients at 0.87 (CI 95% 0.764, 0.965).

DISCUSSION

Liver biopsy plays an important role in the diagnosis and management of complications of chronic hepatitis C infection such as liver fibrosis ((EASL, 1993). There are alternative noninvasive methods for assessing liver fibrosis which have been proven to be as effective as liver biopsy (Castera L, 2008). The most extensively analyzed non-invasive test for the assessment of liver biopsy is the FibroTest which analyzes the serum levels of six biochemical markers of fibrosis namely; γ -glutamyltranspeptidase, alanine-aminotransferase, α 2-macroglobulin, haptoglobin, apolipoprotein A1, and total

bilirubin (Imbert-Bismut F et al, 2001; Poynard T et al, 2002). Non-invasive imaging of liver is another useful tool for the assessment of fibrosis (Yin M et al, 2007; Sandrin L et al, 2003)

In our study, serum levels of Liver enzymes were found to be elevated significantly with the progress of liver fibrosis. The association was more apparent for ALT and gamma-GT. These two serum markers showed significantly higher positive correlation to severe fibrosis. Therefore, the higher the ALT and GGT level, the more advanced the stage of liver fibrosis. This is compatible with the study done by Mathurin et al. (Mathurin P et al., 1998) who found that fibrosis was more progressive in hepatitis C patients with elevated serum ALT level compared to those with normal ALT. In this regard, serum AST level was a less accurate marker of liver fibrosis as it is also released by other organs besides liver such as heart and red blood cells.

This study also identified serum albumin level as a useful marker in the identification of mild (or no) liver fibrosis. This result was expected because albumin is exclusively synthesized by liver, hence progression of liver fibrosis leads to reduced production of albumin by the hepatocytes. Similar results were also obtained by Gomez et al. (Gomez- Dominguez E et al, 2006) who found that serum albumin level was significantly lower in severe fibrosis.

In this study, we also observed a positive correlation between serum HDL level and degree of liver fibrosis. This finding was in agreement with the findings of meta-analysis by (Poynard T et al, 2002; Rosenthal-Allieri MA et al, 2007) that found that HDL had significant value in predicting the absence of or presence of no more than

minimal fibrosis on liver biopsy and in predicting the absence of cirrhosis on liver biopsy with the AUROC at 0.81. The liver synthesized class A apolipoprotein and the lipoprotein is essential for the formation of HDL. As the liver fibrosis progresses to more advanced stages, the ability of the liver to synthesize the lipoprotein decreases and directly reduces the formation of HDL.

Our study also identified serum aldosterone as a useful marker in the stratification of liver fibrosis. Animal studies have shown a role for the renin-angiotensin-aldosterone system (RAAS) in the progression of liver fibrosis (Paizis G et al, 2005). Though the role of RAAS in glomerulosclerosis and myocardial remodeling is well known, its role in liver fibrosis has not been clearly defined (Warner FJ et al., 2007, Töx U et al, 2006). It has been reported that therapeutic inhibition of the renin-angiotensin system by the angiotensin-converting-enzyme (ACE) inhibitor, significantly suppressed liver fibrosis in animal study models (Yoshiji H et al, 2006). A similar role in humans has been postulated (Yoshiji H et al, 2007). Serum aldosterone level and their role in generating liver fibrosis has not been adequately explored by research despite its well established role in glomerular sclerosis and myocardial fibrosis. This study underscores the value of aldosterone in the diagnosis of liver fibrosis.

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