Original Article

Non Invasive Assessment of Liver Fibrosis in Chronic Liver Disease

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Background: Chronic hepatitis C (CHC) infection leads to necroinflammation which causes fibrosis and ultimately cirrhosis in 20% of cases. Liver biopsy is recommended for assessment of hepatic fibrosis and is thought to be the gold standard. Recurrent bouts of hepatocellular necrosis in CHC infection cause episodic rise in aminotransferases especially alanine aminotransferase (ALT). ALT levels decrease as fibrosis advances and AST levels tend to exceed ALT. This results in increase in AST/ALT ratio >1.0, which can be used as non-invasive marker of hepatic fibrosis and cirrhosis, thus reducing the number of liver biopsies performed in patients with AST/ALT ratio >1.0.

Objectives: To assess the accuracy of serum aminotransferases (AST/ALT) ratio for determining the severity of hepatic fibrosis in patients with chronic hepatitis C, considering histopathological findings as gold standard.

Study Design: Cross sectional survey.

Setting: Medical unit 4, Services Institute of Medical Sciences (SIMS)/ Services Hospital, Lahore.

Duration: The study was completed over a period of 6 months; from 15th October, 2008 to 15th April, 2009.

Subject and Methods: Seventy HCV positive with detectable RNA by Polymerase Chain Reaction, fulfilling the criteria were selected. AST and ALT levels were measured in (IU/L). Upper limit of both AST and ALT was taken as 40 IU/L. AST/ALT ratio was calculated as under;
AAR = AST (IU/L), ALT (IU/L) All these patients also underwent percutaneous liver biopsies and then histopathologist staged biopsy for fibrosis according to Ishak/ Knodell criteria.

Results: AST/ALT ratio of more than 1.0 was associated with severe fibrosis/ cirrhosis (F4- f6).

Conclusion: Study showed that AAR more than 1.0 has significant association with severe fibrosis and identifies CHC patients with marked fibrosis/ cirrhosis, and its application can decrease the need for performing liver biopsies for staging.

Key Words: Cirrhosis, CHC, AAR and Fibrosis

Introduction

Hepatitis C virus (HCV) infects an estimated 170 million people worldwide. It is the leading cause of cirrhosis in Pakistan.[1] After acute HCV infection, the likelihood of remaining chronically infected approaches 85 to 90%. Although many patients with chronic hepatitis C have no symptoms, cirrhosis may develop in as many as 20% within 10 to 20 years of acute illness.

The presence of underlying fibrosis identifies the patient who are at risk for continued progression of liver disease and determines the clinical outcome. Therefore an accurate assessment of stage of fibrosis and extent of necroinflammatory activity is essential to guide management and predict prognosis.[2] The Ishak system assesses fibrosis in seven categories, ranging from normal to cirrhosis and so has potentially discriminant descriptive power.[3] All scoring systems basically use the same principles to record liver disease stage. In the original Knodell publication, Histological Activity Index (HAI) was stated to be "numerical, objective, and reproducible in all the cases".[4]

Liver biopsy is recommended for the management of patients infected by hepatitis C virus (HCV) and is currently the gold standard in assessing liver histology. It is an invasive test prone to complications with a morbidity rate of 0.3 to 0.6% and a mortality rate upto 0.05%. In chronic hepatitis C (CHC) infection, a liver biopsy provides important information that guides treatment decisions, but is invasive, expensive and associated with possible complications. These issues provide the rationale for the increasing interest in the use of combination of non-invasive biochemical markers to predict the degree of hepatic fibrosis.[5] These markers are

Inexpensive, permit frequent sampling, and may possibly reduce the number of liver biopsies performed. Although a frequent episodic rise in aminotransferases above the normal value, particularly serum alanine aminotransferase (ALT) reflects recurrent bouts of hepatocellular necrosis in chronic hepatitis C (CHC), there is a direct correlation between increased serum aspartate aminotransferase (AST) levels and portal inflammation. Alanine aminotransferase (ALT) levels decrease as fibrosis advances and once cirrhosis is established, aspartate aminotransferase levels tend to exceed alanine aminotransferase. The resulting increase in AST/ALT ratio (AAR) > 0.8 can identify patients with fibrosis and is one of the earliest indicators of cirrhosis.[6] The AST/ALT ratio is approximately 0.8 in normal subjects. A ratio > 1.0 may suggest the presence of fibrosis and its staging in patients with chronic viral hepatitis. An AST/ALT ratio > 1.0 has 100% specificity and 81.3% sensitivity for cirrhosis and staging degree of fibrosis using corresponding liver biopsy specimens as the gold standard[7]. In this study we intend to measure aminotransferases ratio and use this to determine accuracy in predicting severity of hepatic fibrosis in patients with chronic hepatitis C infection, so that the number of liver biopsies can be reduced in patients with AST/ALT ratio > 1.0.

Objective Of Study

The objective of the study was;

To assess accuracy of serum aminotransferases (AST/ALT) ratio for determining the severity of hepatic fibrosis in patients with chronic hepatitis C, considering histopathological findings as gold standard.

Operational Definitions

Aminotransferases (AST/ALT) Ratio: AST and ALT

were measured in IU/L and their ratio was calculated.

Normally this ratio is < 0.8. Chronic Hepatitis C: Patients, who were HCV positive by Polymerase Chain Reaction (PCR). True +ve: The patients with AST/ALT ratio of >1.0 andhistopathological findings suggestive of stages between fibrosis (F4 F6) was taken as true positive. True ve: The patients with AST/ALT ratio of < 1.0 and histopathological findings suggestive of staging of fibrosis between F0- F3 was taken as true negative.

False +ve: The patients with AST/ALT ratio of >1.0 and fibrosis staging on histopathology as F0-F3 were taken as false positive. False ve: The patients with AST/ALT ratio of < 1.0 and Fibrosis staging on histopathology as F4- F6 were taken as false negative.

Hepatic Fibrosis: Fibrosis was determined by liver biopsy and staging of fibrosis was done from F0 to F6 by Ishak/Knodell criteria as under; F0: No fibrosis (Normal).

F 1: Fibrosis expansion of some portal areas +/- short fibrous septa.

F 2: Fibrous Expansion of most portal areas +/- short fibrous septa.

F 3: Fibrous expansion of most portal areas with occasional portal to portal bridging.

F 4: Fibrous expansion of portal areas with marked bridging as well portal to central.

F 5: Marked bridging with occasional nodules.

F 6: Cirrhosis, probable or definite. F4 to F6 was taken as severe fibrosis/ cirrhosis.

Materials And Methods

Setting

The study was conducted in Medical unit 4 of Services Hospital Lahore, affiliated with Services Institute of Medical Sciences (SIMS), Lahore.

Study Duration

The study was completed over a period of 6 months after the approval of synopsis, from October 15, 2008 to April 15, 2009.

Study Design

It was a Cross-sectional survey.

Sample Size

70 HCV positive patients by PCR were included in the study, based on the selection criteria.

Sampling Technique

Non-probability purposive sampling technique was used.

Sample Selection

Inclusion Criteria

1) HCV positive by Polymerase Chain Reaction (PCR) qualitative .
2) Age between 18-70 years of either sex.
3) Platelet counts > 80000 x109/L on labs.

Exclusion Criteria

1) History of alcohol intake.
2) Co-morbidities of chronic diseases like chronic

clinically.

3) Hepatic Encephalopathy determined clinically.
4) Serum Bilirubin >3.0mg/dl, Serum Albumin <2.8gm/dl, Serum Creatinine >1.5mg/dl on labs.
5) Marked ascites on ultrasonography (USG).

Data Collection

Seventy patients, HCV confirmed on PCR, who fulfilled inclusion and exclusion criteria, were selected from medical unit IV at Services Hospital Lahore after taking informed consent. Each patient was explained the importance and procedure of the study. Serum aminotransferases (AST, ALT) were measured by the same laboratory in IU/L. Aspartate aminotransferases (AST) to Alanine amino-transferase (ALT) ratio (AAR) was calculated and values 0.8 and less were taken as normal. All these patients underwent a percutaneous liver biopsy by the same gastroenterologist on outdoor basis, on the earliest possible date, as were convenient for the patient. Specimens were fixed and stained and sent to same histopathologist on the same day, which assessed for the stage of fibrosis from F0 to F6 according to Ishak/ Knodell criteria (mentioned operationally). Data was entered in the proforma given as Annex. "A" At the end of the study, all the data thus obtained was tabulated and then compared for any association amongst them.

Data Analysis

All the data collected through the proforma was entered in SPSS version 12 and analyzed through its statistical package. Descriptive statistics were calculated. The quantitative variables of study included age, AST, ALT levels and AST/ALT ratio. These variables were presented as mean and standard deviation. The qualitative data included sex and stage of fibrosis. This was presented as percentage. Sensitivity, specificity, positive predictive value and negative predictive value of AST/ALT ratio for the stages of fibrosis were calculated by taking histopathological stages as gold standard by constructing a 2 × 2 table.

Results

Seventy patients who were HCV positive by PCR were selected according to selection criteria from outpatient department of Services hospital Lahore. Variables included age, gender, AST, ALT, AST/ALT ratio and stage of fibrosis. These variables were presented as percentages and counts. The quantitative variables were presented as mean and standard deviation. AST/ ALT ratio and stage of fibrosis were presented in 2×2 table for measurement of sensitivity, specificity, positive predictive value and negative predictive value. Out of total 70 patients 64% were male and 36% were female. Majority of patients (78%) were between ages of 30-60 years. There were few patients at extreme of age groups. Minimum age of patient was 18 years and maximum was 60 years. Mean age was 38.21 with SD 0f 10.53. **Table 1** showed patient according to different stages of fibrosis. Fibrosis was divided into seven categories ranging from F0 to F6. For study purpose, fibrosis was further divided into two subgroups. Those with fibrosis stage F4-F6 were grouped as "severe fibrosis" and with fibrosis stage F0-F3 as "not severe". **Table 1** showed that 23% of patients had no fibrosis as F0. Majority of patients (88.6%) do not show severe fibrosis (F0-F3). Only (11.4%) of patients had severe fibrosis (F4-F6).

Table-1: Distribution of subjects according to stage of fibrosis.

Stage of Fibrosis "F"	Frequency	Percentage
F0	16	22.9%
F1	22	31.5%
F2	19	27.1%
F3	5	7.1%
F4	6	8.60%
F5	1	1.4%
F6	1	1.4%
Total		100.0%

F = Fibrosis
It was graded by Ishak/ Knodell scoring system.
F0-F3 was taken as "not severe" fibrosis.
F4-6 was taken as "severe fibrosis".

Table-2: Distribution of subjects according to aspartate aminotransferase to AST/ ALT ratio.

	Frequecny	Percentage
AST/ALT<1.0	57	81.1%
AST/ALT 1.0	13	18.6%
Total	70	100.0%

Table-3: Association of AST/ ALT ratio with stage of fibrosis.

AST/ALT Ratio	Stage of Fibrosis Frequecny	Not Severe	Total
>1.0	7	6	13
<1.9	1	56	57
Total	8	62	70

Sensitivity= 87.5%
Specificity= 90.3%
Positive predictive value= 53.8%
Negative predictive value= 98.2%

So, in this study it was observed that AST/ALT ratio >1.0 is significantly associated with severe fibrosis (F4-F6) with high sensitivity and specificity

CHC infection causes persistant injury to the hepatocytes and this persistant injury can lead to fibrosis and ultimately to cirrhosis in a considerable number of patients.

Fibrosis is defence mechanism of human body which resembles wound healing. It occurs in response to injury and inflammation[8]. But if this injury remains there for a longer duration, it leads to increased fibrosis and the process becomes pathological. Progression of hepatic fibrosis occurs at different rates in different people. It is more common in men, advancing age, increase duration of disease and other factors like immunosuppression and co-morbidities[9].

If hepatic fibrosis is diagnosed at an early stage, means if it is not severe (F4-F6), it can potentially prove to be reversible with effective treatment, as opposed to once thought that fibrosis is an irreversible phenomenon. A number of studies have been carried out to see effects of treatment which proved that fibrosis is reversible even in severe cases.

Fibrosis is best diagnosed with the help of a liver biopsy which is gold standard to exactly know the stage of fibrosis. Many histological scoring systems are in use but best used now are Ishak system (HAI) and METAVIR scoring system. Ishak system assesses fibrosis in seven stages, ranging from no fibrosis as F0 to cirrhosis as F6[10]. In the past, liver biopsy was thought to be a "MUST" for initiation of treatment, monitoring treatment and to assess prognosis. This lead to increase in number of liver biopsies performed in patients with CHC infection.

Liver biopsy is an invasive procedure which is expertise dependent, costly, associated with risks, complications and chances of mortality. Pre-procedure workup is also required. Patient consent is sometimes an issue for repeated number of biopsies required before starting treatment, during treatment to see response and sometimes in follow up cases after treatment.

Also there are issues with biopsy samples such as size of sample. Adequacy of size of sample is necessary for exactly assessing stage of fibrosis. A study showed that when using METAVIR scoring system, at least 25mm specimen is necessary for assessing stage of fibrosis. Another limitation of liver biopsy is that different areas of liver can be affected differently. So, sampling errors may occur and liver biopsy staging is also observer dependent[11].

Keeping in view all above factors, it has always been felt that a non-invasive method for prediction of hepatic fibrosis is needed. Such a test should be simple, cheap, readily available, reproducable, requiring no expertise, avoiding complications of biopsy and routinely done in labs with comparable accuracy which can reduce if not completely abolish the need for liver biopsy.

Last decade was a decade of research and advances in medical sciences. Many studies were carried out in the last decade to see and find out such non-invasive biochemical tests for prediction of hepatic fibrosis/ cirrhosis in patients with chronic hepatitis C infection[12].

These studies used many biochemical markers including platelet count, AST, AST/ALT ratio, INR, APRI, serum albumin and fibrinogen levels as predictor of fibrosis[13].

Giannini EG, et al used AST/ALT ratio in assessing disease severity and prognosis in patients with CLD due to CHC infection. The study showed that AST/ALT ratio >1.0 had 100% specificity, 53% sensitivity, 100% PPV and 81% NPV for cirrhosis using corresponding liver biopsy specimen as gold standard.

Another study was conducted by Mangound AM and colleagues for non histological assessment of liver fibrosis in HCV infection. AST/ALT ratio was assessed with liver biopsy as gold standard. Results showed that AST/ALT ratio has 92.6% sensitivity and 94.3% specificity in predicting severe fibrosis/ cirrhosis. Although sensitivity didn't reach 100%, however its use can reduce number of liver biopsies when diagnosing and treating patients with CHC infections.

Keeping in view the above mentioned facts, AST/ALT ratio was used in our study to identify the stage of fibrosis as severe (F4-F6) and not severe (F0-

AST/ALT ratio was found to be highly sensitive (87.5%) and specific (90.3%) with a high negative predictive value (98.2%).

Comparison was done with other studies conducted in the past. Park G and colleagues conducted a similar study which showed similar results.

The best thing of AST/ALT ratio was its simplicity, availability, being non-invasive, inexpensive and requiring no expertise.

Other advantages included, avoidance of complications of biopsy and routinely done in labs with comparable accuracy. Another advantage could be its use in areas where liver biopsy facilities are not available, as in most parts of our country, as a predictive factor of hepatic fibrosis.

A shortcoming of this study was that, because of very strict selection criteria, only patients with compensated disease were included in this study. This resulted in selection of less number of patients with decompensated disease, that didn't allow us to see whether these variables have any effect on results of study.

Second disadvantage may be a low positive predictive value (53.8%). Another disadvantage may be that study results needs validation and validation requires longitudinal studies.

So, the ultimate use of a non-invasive test for fibrosis prediction in CHC infection depends on validation by further studies. Until that, these non-invasive biomarkers of hepatic fibrosis can be used as qualitative test but not as quantitative one.

Conclusion

Our study showed that a simple biochemical test, AST/ALT ratio, that is non-invasive, cheap, easily available and reproducible, as a significant association with presence or absence of severe fibrosis (F4-F6) in patients with CHC infection. AST/ALT ratio has 87.5% sensitivity, 90.3% specificity, 98.2% NPV and 53.8% PPV. The study showed high degree of accuracy for prediction of hepatic fibrosis as severe (F4-F6) and not severe (F0-F3).

AST/ALT ratio needs further longitudinal studies on large number of patients in order to validate its use.

If its accuracy is confirmed, it may be used as non-invasive marker of staging hepatic fibrosis as severe (F4-F6) and not severe (F0-F3). This will help in reducing if not completely abolishing need for liver biopsy, especially in areas where expertise for liver biopsy are not available, as in most areas of Pakistan.

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