Original Article

Use of Serum Aspartate Aminotransferase Levels and Platelet Count to Predict Hepatic Fibrosis in Chronic Hepatitis C

Sajid Nisar, Fehmida Farrukh Khan and Faisal Masud

Background: Chronic hepatitis C (CHC) induces inflammation resulting in fibrosis. Liver biopsy is the gold standard for assessing histology. Progressive fibrosis results in portal hypertension, splenomegaly, thrombocytopenia and decreased clearance with rise of the enzyme serum aspartate aminotransferase (AST) levels. To amplify this difference in AST and platelet count in fibrosis, AST-platelet-ratio-index (APRI) was devised using noninvasive serum markers, suggesting that its application may decrease the need for liver biopsy.

Patients and Methods: Cross sectional descriptive study done in sixty HCV positive patients fulfilling the criteria. AST levels (IU/L) expressed as a ratio of upper limit of normal (ULN) taken as 40, were divided by platelet count ($x10^9$/L) and multiplied by 100 to calculate

APRI = {(AST/40)/Platelet Count} x 100.

Liver biopsies were then staged by histopathologist for fibrosis according to Ishaq /revised Knodell criteria.

Results: APRI of less than 1.5 was associated with absent or minimal fibrosis (F0-F2), whereas values greater than these showed marked fibrosis/ cirrhosis (F3-F6) (p=0.0001).

Conclusion: Study showed that APRI has significant association with fibrosis and identifies CHC patients with minimal as well as marked fibrosis and its application may decrease the need for performing liver biopsies for staging.

Key words: CHC, Fibrosis, Cirrhosis, APRI

Introduction

Hepatitis C virus (HCV) infects an estimated 170 million people world wide.[1] It has emerged as nature's leading cause of both chronic hepatitis and cirrhosis.[2] Up to 80% of patients with acute hepatitis C eventually develop chronic hepatitis, where inflammation and necrosis continue for at least 6 months. In fact CHC is the leading cause of cirrhosis in Pakistan.[3]

Despite its chronicity, majority of patients with CHC may remain asymptomatic with no sequelae.[4] However the progressive form of the disease with extensive fibrosis ultimately leads to cirrhosis in 20% of patients in over 10-20 years.[5]

Fibrosis occurs at variable rates in different patients, it involves formation of septae, nodules and architectural reorganization.[6]

The stage of fibrosis is thought to identify the patient who is at risk for continued progression of liver disease. It is one of the main determinants of the clinical outcome, influencing decisions regarding antiviral treatment.[7]

Although fibrosis is reversible in its initial stage, the exact point where it becomes irreversible is incompletely understood. Increasing evidence suggests that even early stages of cirrhosis may be reversible.[8] Thus an accurate assessment of stage of fibrosis is essential to guide management and predict prognosis.[9] Advanced cirrhosis represents the end stage of any chronic liver disease.[10] It was the 12th leading cause of death in the United States in 2000, accounting for more than 25,000 deaths.[11] It eventually leads to two major syndromes of portal hypertension and hepatic insufficiency.[12]

Patients and Methods

The study was conducted in Medical Unit 4, Services Institute of Medical Sciences, Lahore. The study was completed over a period of 7 months between September 1, 2006 and March 31, 2007. It was a cross-sectional descriptive study.

Inclusion Criteria

1. HCV positive by Polymerase chain reaction (PCR).

2. Age between 12 to 70 years of either sex.
3. Platelet count >80,000.

Exclusion Criteria

1. Serum Bilirubin >3.0mg/dl, Serum Albumin < 2.8 gm/dl, Serum Creatinine> 1.5mg/dl.
2. Co-morbidities of any chronic disease like congestive heart failure, chronic renal failure.
3. Hepatic Encephalopathy.
4. History of Alcohol intake.
5. Marked ascites on ultrasonography.

Statistical Analysis

Data was entered on SPSS version 10 and analyzed accordingly. The variables that were analyzed included demographic (age, sex) and routine investigations (liver function tests, blood biochemistry which included platelet counts). The measured variables were presented as mean and standard deviation and converted data was presented as proportions. The specific investigations and APRI were presented as frequency distribution tables. The data was correlated for the APRI index and other variables like stage of fibrosis which was the indicator of stage of pathology. Any association observed was subjected to Chi Square test as the variables were converted into nominal groups. $p<0.05$ was considered significant. Phi & Cramer's V statistics were used to predict the degree of association of significant factors with fibrosis

Results

A total of 60 HCV positive patients by PCR were selected for the study. They were made comparable with each other with regards to demographic variables (age, sex) and other laboratory investigation related factors like platelet count and aspartate aminotransferase levels and stage of pathology. Data was analyzed descriptively and analytically. Amongst the 60 patients, 67% were males and 33% were females (**Table 1**). Overall percentage of males was more than that of females.

Table-2 shows age distribution of patients. According to it, majority of HCV positive patients belonged to age group 41-60 years (73%). There were few patients at the extremes of age groups shown, with the minimum age of 16 & maximum age of 55 years. Mean age was 37.17 ± SD of 9.35 years (CI= 95%).

Table-1: Distribution of subjects according to gender.

Gender	Frequency	Percentage
Male	40	66.7
Female	20	33.3
Total	60	100.0

Table-2: Distribution of subjects according to age.

Age in years	Frequency	Percentage
Less than 25	6	10.0
26-35	22	36.6
36-45	21	35.0
More than 45	11	18.3
Total	60	100.00
Mean ±SD	37.17±9.36	

Table-3: Distribution of subjects according to stage of fibrosis.

Stage of Fibrosis F[1]	Frequency	Percentage
F0	14	23.3
F1	20	33.3
F2	17	28.3
F3	6	10.2
F4	2	3.3
F5	0	0.0
F6	1	1.7
Total	60	100

F[illegible] minimal fibrosis, F2 moderate fibrosis, F3 marked fibrosis, F4-6 severe fibrosis

Fibrosis was staged in six categories , ranging from F1 to F6. For convenience fibrosis was further divided into two groups; those with absent or minimal fibrosis were grouped under stage F1- F2 belonging to early stage whereas those with marked fibrosis were grouped under stage F3-F6. Table 3 shows that 23% of patients had no fibrosis (F0), whereas majority of patients (62%) showed minimal to moderate fibrosis and belonged to early stage represented by stages F1-F2. It was noticed that only a few patients (15%) showed marked fibrosis/cirrhosis stages (F3-F6).

Another significant independent variable was found to be AST levels (IU/L) expressed as ratio of upper limit of normal (AST/ULN) where ULN was taken as 40 (**Table 4**).

82% of patients showed decreased levels of AST< 2, 18% having values > 2, showing that most of the patients had AST values of less than twice the upper

limit of normal **(Table 4)**. Platelet counts were on the higher side with more than 85% counts > 150($x10^9$) only 5% < 150($x10^9$) **(Table 5).**
Similarly APRI values of < 0.5 were 47%, those between 0.6-1.5 were 42% and only 11% values were > 1.5. In Table7, Fisher's exact test was applied which showed that both variables were significantly associated. The value of chi-square was found to be 9.789 with p value of 0.007.
These results suggest that AST is significantly associated with fibrosis. The value of Cramer's V statistic is 0.404 which shows that variables are positively associated. In Table8, again Fisher's exact test was applied. The value of chi-square was 7.200 with p value of 0.022. These results suggest that platelet counts are significantly associated with fibrosis. The value of Phi statistic is -0.364 which shows that variables are negatively associated.
The cumulative effect of independent variables AST and platelet counts was noticed in the APRI index which showed that values less than 0.5 have no fibrosis (55%) and none of them showed marked or severe fibrosis. APRI values between 0.6-1.5 showed that 43% had absent or minimal fibrosis, whereas 33% showed marked fibrosis. Among patients with

Table-4 : Distribution of subjects according to Aspartate Amino-transferase/ upper limit normal ratio (AST/ ULN) (n=60)

	Frequency	Percentage
AST/ULN[1]<2	49	81.7
AST/ULN ≥2	11	18.3
Total	60	100.0

[1]AST/ ULN=Aspartate aminotransferase/ upper limit of normal (Normal value of AST is taken as 40 IU/L)

Table-5 : Distribution of subjects according to Platelet count (n=60)

	Frequency	Percentage
Platelets ≤ 150 (10^9/L)	9	
Platelets > 150 (10^9/L)	11	
Total	60	100.0

Table-6: Distribution of subjects according AST to Platelet Ratio Index (APRI) (n=60)

	Frequency	Percentage
APRI[1] <0.5	28	46.7
APRI 0.6-1.5	25	41.7
APRI >1.5	7	11.7
Total	60	100.0

** APRI=Aspartate Aminotransferase to Platelet Ratio Index.*

Table-7: Association of aspartate aminotransferase level/ upper limit normal (AST/ ULN) with stage of fibrosis.

Stage of fibrosis	AST/ULN<2	AST/ULN≥ 2	Total
None or minimal	45(88.02%)	06(11.76%)	51(100%)
Marked	04(44.44%)	05(55.55%)	09(100%)
Total	49(81.66%)	11(18.33%)	60(100%)

Chi-square 9.798
Cramer's V statistic 0.404
Df 1
P value 0.007
AST/ ULN =Aspartate aminotransferase/ upper limit of normal (normal value of AST is taken as 40 IU)

Table-8: Association of platelet count with stage of fibrosis (n=60)

Stage of fibrosis	Platelet count (10^9/L) ≤ 150	> 150	Total
None or minimal	05(09.80%)	46(90.19%)	51(100%)
Marked	04(44.44%)	05(55.55%)	09(100%)
Total	09(15%)	51(85%)	60(100%)

Chi-square 7.200
Df 1
P value 0.022
Phi statistic -0.364

Table-8: Association of APRI with stage of fibrosis (n=60)

Stage of fibrosis	Asparate aminotransferase to platelet ratio index (APRI) APRI <0.5	APRI 0.6-1.5	APRI >1.5	Total
None or minimal	28 (54.90%)	22 (43.13%)	01 (01.96%)	51 (100%)
Marked	0 (00%)	03 (33.33%)	06 (66.66%)	09 (100%)
Total	28 (46.66%)	25 (41.66%)	07 (11.66%)	60 (100%)

Chi-square = 32.571, Df = 2, P value = 0.000

APRI values > 1.5, only a few (2%) had absent or minimal fibrosis whereas majority (67%) had marked fibrosis/cirrhosis.

In the descriptive analysis it was found that mean age of the patients was 37.17 years with 95% CI 34.75 to 39.67.

In this study it was found that some factors like AST, platelets and APRI are significantly associated with fibrosis. Moreover, AST and APRI are positively associated while platelets are negatively associated.

Conclusion

In conclusion, this study showed that a simple index, the APRI, consisting of 2 readily available laboratory results, AST levels and platelet count, shows significant association with absence or presence of fibrosis in treatment naive CHC patients with a high degree of accuracy .The APRI can be determined in the clinic or at the bedside. Using one simple formula, the absence or presence of significant fibrosis can be accurately assessed in up to 90% and 60% of CHC patients respectively, potentially avoiding the need for liver biopsy in these patients. Futher studies are needed to validate the APRI in a larger number of CHC patients, particularly in community based programs and daily practice. More attention needs to be paid to adequacy of biopsy samples obtained, as well as reliability of assessment of fibrosis for correlation with the non invasive serum markers. This factor has marked clinical implications .It is important with regards to diagnosis of future patients, in order to avoid failure to recognize presence or absence of fibrosis in patients who may be inappropriately treated, or denied treatment on these bases. It may also have a direct effect on predicting prognosis and course of the disease.

Department of Medicine
Services Institute of Medical Sciences, Lahore.
theesculapio@hotmail.com

References

1. Bronowicki JP, Barraud H, Peyrin BL. Epidemiology and natural history of hepatitis C. Rev Prat 2005; 55 :607-14.
2. Garcia TG. Current management of the complications of cirrhosis and portal hypertension. Gastroenterol 2001; 120: 726-48.
3. Nadeem MA, Waseem T, Sheikh AM, Grumman N, Irfan K, Hasnain SS et al. Hepatitis C an alarming increasing cause of Liver Cirrohsis in Pakistan. Pak J Gastroenterol 2002; 16:3-8.
4. Deinstag JL. Acute viral hepatitis complications and sequel. Harrisons's principles of internal medicine. 15th ed .New York: McGraw-Hill Companies ;1998. 2074.
5. Friedman LS. Liver and biliary tract and pancreas, chronic viral hepatitis. Current medical diagnosis and treatment.46th ed. New York: McGraw Hill Company 2007; 675.
6. Crawford JM. The liver and biliary tract. Robin's basic pathology. 7th ed. New York: W.B. Saunders Company 2003;393.
7. Dienstage JL. The role of liver biopsy in chronic hepatitis C. Hepatol 2002; 36: 152-60.
8. Bonis PA, Friedman SL, Kaplan MM. Is liver fibrosis reversible. N Engl J Med 2001; 344: 452.
9. Ghany MG, Kleiner DE, Alter H, Doo E, Khokar F, Promrat K et al. Progression of fibrosis in chronic hepatitis C. Gastroenterol 2003; 124: 97-104.
10. Fernandez EG, Sanchez FA, Gines P. A prognostic model for predicting survival in cirrhosis with ascites. J Hepatol 2001; 34: 46-52.
11. Anderson RN. Deaths: leading causes for 2000. National vital statistics reports. Vol. 50 No.16. Hyttoville MD, National center for health statistics 2002. publication no. 2002-112002-0522.
12. DeFranchis R, Pascal JP, Burroughs AK, Henderson JM, Fleig W, Groszmann RJ et al. Definitions, methodology and therapeutic strategies in portal hypertension. J Hepatol 1998; 15: 256-61.
13. Claassen J. The gold standard not a golden standard. BMJ 2005; 330: 1121.
14. Knodell RG, Ishak KG, Black WC. Formulation and appli- cation of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatol 1998; 1:431-5.
15. Ishak K ,Baptista A, Bianchi L, Callea F, DeGroete J, Gudat F et al. Histologic grading and staging of chronic hepatitis. J Hepatol 1998; 22: 696-9
16. Cadranel JF, Rufat P, Degos F. Practices of liver biopsy in France: results of a prospective nationwide survey. Hepatol 2000;32(3):477-81.
17. Regev A, Berho M, Jeffers L J, Millikowski C, Molina EG, Pyrsopoulos NT et al. Sampling error and intra observer variation in liver biopsy in patients with chronic HCV infection. Am J Gastrenterol 2002; 97: 2614-8.

18. Lok AS, Ghany MG, Goodman ZD, Wright EC, Everson GT, Sterling RK et al. Predicting cirrhosis in patients with hepatitis C based on standard laboratory tests: results of the halt-cohort. Hepatol 2005 ;42 :282-92.
19. Imbert-bismut F, Ratziu V, Peironi I, Charlotte F, Benhamou Y, Poynard T et al. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: prospective study. Lancet 2001; 357:1069-75.
20. Pohl A, Behling C, Oliver D, Kilani M , Monson P, Hassanien T . Serum amino transferase levels and platelet counts as predictors of degree of fibrosis in chronic hepatitis C virus infection. Am J Gastroenterol 2001; 96:3053-5.
21. Kapplan MM. Alanine amino tranferase levels : what's normal? Ann Intern Med 2002 ;137:49-51.
21. Bacon BR. Treatment of patients with hepatitis C and normal serum amino transferase levels. Hepatol 2002; 36: 179 -84.
22. Assy N, Minuk JY. Serum aspartate but not alanine amino-transferase levels help to predict the histological features of chronic hepatitis C viral infection in adults. Am J Gastroenterol 2000; 95: 1545-50.
23. Kamimoto Y, Horiuchi S, Tanase S, Morino Y. Plasma clearance of intravenously injected aspartate amino tranferase isozymes: evidence for preferential uptake by sinusoidal liver cells. Hepatol 1998;55:367-75.
24. Giannini E, Borro P, Botta F, Fumagali A, Malfatti F, Podesta E et al. Serum Thrombopoietin levels are linked to liver function in untreated patients with hepatitis C virus related chronic hepatitis. J Hepatol 2002; 37: 572-7.
25. Butt AR, Ahmed S, Haider N, Ditta A, Khokhar AS, Khokhar MS et al. Evaluation of AST /ALT ratio in patients with liver disease. Pak J Med Sci 2001; 17: 225-9.

Answer Picture Quiz

A1 Scar on the neck suggestive of thyroidectomy.
A2 Hypoparathyroidism secondary to iatrogenic removal of the parathyroid glands along with the thyroid gland (a common complication of the surgical procedure).
A3 The patient developed carpopedal spasm also called tetany secondary to hypocalcemia.
A4 The patient was administered IV Calcium.
A5 To prevent future spasms the following steps are to be taken:

- Oral Vitamin D3 and Calcium supplementation
- High Calcium, low Phosphate diet
- Regular monitoring of serum Calcium levels
- Teriparatide (a synthetic analogue of PTH) may be treatment of choice in future