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

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ABSTRACT

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 counts in fibrosis, AST-platelet-ratio-index (APRI) was devised using noninvasive serum markers, suggesting that its application may decrease the need for liver biopsy. **Material and methods:** Cross sectional descriptive study done insixty patients of HCV positive state fulfilling the criteria were selected. AST levels (IU/L) expressed as a ratio of upper limit of normal (ULN) taken as 40, were divided by platelet counts($x10^9$/L) and multiplied by 100 to calculate

$$APRI = \frac{AST/40}{\text{Platelet count}} \times 100$$

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=.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 natures leading cause of both chronic hepatitis C (CHC) 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 after the, from September 1, 2006 to March 31, 2007. It was a Cross-sectional descriptive study.

Inclusion criteria

1. HCV positive by Polymerase chain reaction (PCR).
2. Age between 12 – 70years 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. Comorbidities 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

All data was entered on SPSS.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 investigation and APRI were presented as frequency distribution table. 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.

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 variables demographic (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. In our descriptive statistics we found out counts and percentages of each variable.

In our analytic section we carried out a bivariate analysis. Chi-Square test was used to measure the association of different factors with fibrosis. Phi & Cramer's V statistic were used to predict the degree of association of significant factors with fibrosis.

Amongst the 60 patients 67% were male and 33% were females (Table 1). Overall percentage of males was more than that of females.

Table 1: Distribution of subjects according to their gender (n=60)

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

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

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 grouped under stage F3-F6.Table 3 showed 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).

Table 2: Distribution of subjects according to their age (n=60)

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	
Minimum age	16 years	
Maximum age	55 years	

Table 3: Distribution of subjects according to stage of fibrosis (n=60)

Stage of Fibrosis F[1]	Frequency	Percentage
F 0	14	23.3
F 1	20	33.3
F 2	17	28.3
F 3	6	10.2
F 4	2	3.3
F 5	0	0.0
F 6	1	1.7
Total	60	100

[1]F = fibrosis as graded by Ishaq scoring i.e. F0 no fibrosis, F1 minimal fibrosis, F2 moderate fibrosis, F3 marked fibrosis, F4-6 severe fibrosis

Table 4: Distribution of subjects according to Aspatate Aminotransferase/ upper limit normal (AST/ ULN) ratio (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 IU0)

Another significant independent variable was found to be AST levels (IU/L) expressed as ratio of upper limit of normal (AST/ULN 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% with counts > 150(x10 ^{9}) only 5% < 150(x10 ^{9}) (Table 5).

Similarly APRI values of <.5 were 47%, those between .6-1.5 were 42% and only 11% values were > 1.5.

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

	Frequency	Percentage
Platelet ≤150 (10^9/L)	9	15.0
Platelet >150 (10^9/L)	51	85.0
Total	60	100.0

Table 6: Distribution of subjects according aspartate aminotransferase to Platelet Ratio Index (APRI)

	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

[1] APRI Aspartate Aminotransferase to Platelet Ratio Index

In Table 7, we made a cross table for the analysis of chi-square. We observed that the frequency of one cell is less than 5. As our table is 2x2 we found an alternative way to calculate chi square is Fisher's exact test which shows that both variables are significantly associated. The value of chi-square was found to be 9.789 with p-value=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 the Table 8, we made a cross table for the analysis of chi-square. Again Fisher's exact test was applied. The value of chi-square was 7.200 with p-value=0.022. These results suggest that platelets are significantly associated with fibrosis. The value of Phi statistic is -0.364 which shows that variables are negatively associated.

Table 7: Association of aspartate aminotransferase level/ upper limit normal (AST/ ULN) with stage of fibrosis (n=60).

Stage of fibrosis	Aspartate aminotransferase level / upper limit normal (AST/ ULN)		Total
	AST/ ULN < 2	AST/ ULN ≥ 2	
No or minimal fibrosis	45(88.2%)	6(11.76%)	51(100%)
Marked fibrosis	4(44.44%)	5(55.55%)	9(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		

[1] AST/ ULN = Aspartate aminotransferase / upper limit of normal (normal value of AST is taken as 40 I.U)

Table 8: Association of platelet count with stage of fibrosis/

Stage of fibrosis	Platelet count (10^9/L)		Total
	≤150	> 150	
No or minimal fibrosis	5(9.80%)	46(90.19%)	51(100%)
Marked fibrosis	4(44.44%)	5(55.55%)	9(100%)
Total	9(15%)	51(85%)	60(100%)

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

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 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 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. More over AST and APRI are positively associated while platelets are negatively associated.

Table 9: Association of APRI with stage of fibrosis (n=60)

Stage of fibrosis	Aspartate Aminotransferase to Platelet Ratio Index APRI			Total
	APRI < 0.5	APRI 0.6-1.5	APRI >1.5	
No or minimal fibrosis	28 (54.90%)	22 (43.13%)	1 (1.96%)	51 (100%)
Marked fibrosis	0(00%)	3 (33.33%)	6 (66.66%)	9 (100%)
Total	28 (46.66%)	25 (41.66%)	7 (11.66%)	60 (100%)

Chi-square	32.571
Df	2
P value	0.000

[1] APRI = Aspartate Aminotransferase to Platelet Ratio Index

DISCUSSION

Histological assessment by liver biopsy is considered the gold standard.[13] Currently the most widely used system is the Ishak "revised Knodell"system,[14] whereby disease activity is numerically scored using "The Histological Activity Index" (HAI), which grades CHC as mild, moderate, or severe. It also measures the degree of fibrosis by grading fibrosis in seven categories as follows, 0 = none, 1 = mild, 2 = minimal/moderate, 3 = marked, 4 – 6 = severe/cirrhosis. [15]

Percutaneous liver biopsy is generally considered a safe procedure even though it is invasive, costly, and carries a small risk of complications. [16] In addition there may be sampling errors, inter observer discrepancies, and under staging of cirrhosis.[17] Considering these issues there has been an increasing effort to identify "serum markers" as a non invasive measure to predict hepatic fibrosis.[18] Various combinations of biochemical serum markers have been used. These are inexpensive, permit frequent sampling, and may possibly reduce the number of liver biopsies performed. [19]

These markers are associated with matrix deposition and degradation. Some are cytokines and chemokines associated with fibro genesis, while

others are routine laboratory tests, like serum aminotransferases and platelet counts. [20]

Although frequent episodic rises of serum alanine aminotransferases (ALT) reflects recurrent bouts of hepatocellular necrosis in CHC, they do not correlate with degree of severity of liver desease.[21] In fact serum ALT levels tend to fall as fibrosis advances.[22]

A direct correlation exists between serum aspartate aminotransferase (AST) and portal inflammation.[23] Progression of fibrosis results in decreased clearance and hence increased AST levels. [24]

Reduced platelet counts, which are the result of increased sequestration in the spleen,and decreased thrombopoitin levels [25] along with an increase in AST/ALT ratio,[26] have also been identified as the most significant predictors of marked fibrosis, and one of the earliest indicators of cirrhosis.

Considering this the aim of our study was to construct a simple index AST to platelet ratio index (APRI),find its association with fibrosis on biopsy, and asses its accuracy in identifying the stage of fibrosis and cirrhosis among CHC patients.

CONCLUSION

In conclusion my study showed that a simple index the APRI, consisting of 2 readily available laboratory results AST levels and platelet counts, shows significant association with absence or presence of fibrosis in treatment naïve 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 upto 90% and 60% of CHC patients respectively, potentially avoiding the need for liver biopsy in these patients. Further studies are needed to validate the APRI in a larger number of CHC patients, particularly in community based programmes and daily practices. 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 basis. It may also have a direct effect on predicting prognosis and course of the disease.

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