ORIGINAL ARTICLE

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Accuracy of Biochemical Markers and Platelet Count for Diagnosis of Liver Fibrosis Staging in Patients with Liver Fibrosis, Loghman Hakim & Taleghani Hospitals, Tehran, Iran (2000-2004)

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Background and Aims: **Liver biopsy is the best known technique for evaluation of liver fibrosis. However, alternative diagnostic methods have been investigated to replace this invasive procedure. Our aim was to assess the diagnostic performance of platelet count and biochemical markers for the staging of liver fibrosis in our patients.**

Methods: **In a descriptive study, records of all consecutive patients who underwent liver biopsy at Loghman Hakim & Taleghani hospitals between March 2000 and March 2004 were retrospectively studied. The clinical and laboratory data were obtained from patients' clinical records. Liver histology samples were reviewed by an expert pathologist. The platelet count, serum albumin levels, and the ratio of serum aspartate aminotransferase (AST) levels to platelet count were used as markers for fibrosis. Cutoff points for some of these markers were established using the ROC curve.**

Results: **One hundred thirty patients (94 males & 36 females) were studied. The AST/platelet ratio at the cutoff point 0.39 had a positive predictive value (PPV) of 0.70 and a negative predictive value (NPV) of 0.54 to differentiate patients without liver fibrosis from those with moderate fibrosis. This ratio had a PPV = 0.76 and NPV = 0.70 to differentiate patients without liver fibrosis from those with severe fibrosis when used at a cutoff point of 0.25. The platelet count at the cutoff point of 158500 was used for the distinction of mild from moderate fibrosis. The platelet count at the cutoff point of 151000 and serum albumin at the cutoff point of 3.6 were used for distinction of mild from severe fibrosis. No single marker was found to diagnose different stages of fibrosis.**

Conclusions: **The AST/platelet ratio had an appropriate cutoff point to differentiate patients without liver fibrosis from patients with moderate to severe liver fibrosis. The clinical utility of these tests requires further investigation.**

Keywords: **Liver Fibrosis, Biochemical Markers, Platelet Count, Cirrhosis**

Introduction

Hepatic fibrosis is a common consequence of several chronic liver diseases which may lead to death [1, 2]. Histopathologic changes such as presence of regenerative nodules surrounded by connective tissue are the basis of diagnosing fibrosis and its advanced form, cirrhosis. The developed fibrosis will be irreversible even when the initial cellular injury which caused the regeneration and fibrosis process stops. A variety of diseases including infections, metabolic diseases, drugs and sarcoidosis may lead to hepatic fibrosis [3]. Estimation of fibrosis degree and assessing therapy in chronic liver disease requires assessment of specific variables, the most

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common of which are called fibrosis markers [4-11].

Despite its invasiveness and potential complication such as hemorrhage, pneumothorax, colon and gallbladder perforation, even in the hands of a trained expert, liver biopsy has remained the gold standard method for studying hepatic inflammation and fibrosis. It's worth mentioning that serial liver biopsies in short intervals for assessing the response to therapy are not cost effective and practical [12, 13]. Other disadvantages of liver biopsy include its cost and the anxiety or stress which it causes for the patient. Studies have shown that liver biopsy has only 80% precision in staging hepatic fibrosis and up to 30% of cases of advanced fibrosis remain undiagnosed with this method [14]. Even when the biopsy is taken by an expert and the sample is studied by an expert pathologist in this field, the stage of fibrosis is reported incorrectly in more than 20% of cases [15].

Many researchers in the past decade have introduced single or multiple noninvasive tests as replacements for liver biopsy. A good marker for evaluating the presence or absence of fibrosis must be sensitive, specific and have high positive and negative predictive values. The test must be reproducible, with little in laboratory variations, usable with different etiologies of liver diseases, cheap and easy to use. Not only must it be reliable for staging liver fibrosis but it also must be reliable when the progress of the disease and response to antiviral therapy is being investigated [12, 13]. The quest for finding such a test has started from biochemical markers. A number of these markers are being investigated; some of them are being studied in combination with other biochemical markers along with clinical evaluations [16]. Although no markers alone have the precision to assess hepatic fibrosis, using them in combination with each other has shown promising results [17]. The aim of this study was to assess the accuracy of biochemical factors and platelet count for the diagnosis of liver fibrosis staging.

Materials and Methods

In this diagnostic study, the pathology reports of all liver biopsies from 130 patients [94 males (72.3%) and 36 females (27.7%)] without any exclusion criteria admitted to Loghman and Taleghani hospitals, between 2000 and 2004 were studied. The specimens were reviewed and based on the ISHAK scoring system [3], which stages fibrosis from F0 to F6, the stage of fibrosis was determined. For sake of simplicity, F1 and F2 were considered mild fibrosis; F3 and F4, moderate fibrosis; and F5 and F6, severe fibrosis.

Age, gender, platelet count, levels of albumin, total bilirubin, cholesterol and liver aminotransferases of these patients were determined using their medical records. The obtained data were analyzed using SPSS 11.5 software with the aid of chi-square or Fisher exact tests and Student's t-test for qualitative and quantitative variables, respectively. Cutoff points for some of these markers were established using the ROC curve. P values less than 0.05 were considered significant.

Results

The pathology reports and the markers levels were compared with each other. Twenty-six patients (6 females & 20 males) didn't have any level of fibrosis. Forty-five patients (14 females & 31 males) had mild fibrosis, 31 (5 females & 26 males) had moderate fibrosis and 28 (11 females & 17 males) had severe fibrosis. Thirty patients (23.1%) had HBV infection, 47 (36.1%) had HCV infection, 4 (3.1%) were both HBV and HCV positive, and 4 (3.1%) had autoimmune hepatitis. One patient (0.7%) had alcoholic fibrosis, 5 (3.8%) were diagnosed with malignant liver disease, 2 (1.5%) had tuberculosis, 1 (0.7%) due to prolonged fever, 1 (0.7%) due to Wilson disease and 1 (0.7%) due to lymphoma had undergone liver biopsy. For the other 34 (26.1%) patients, no final diagnosis was documented.

The prevalence of fibrosis between male and female subjects didn't have statistically significant difference. Our data showed statistically significant relationship between age and the degree of fibrosis, demonstrating that the degree of fibrosis increased as the age of patients increased. The mean values of variables are shown in Table 1. None of the studied variables had a statistically significant difference between the patients without fibrosis and those with mild fibrosis; but the AST/platelet ratio ($P<0.03$) and absolute platelet count ($P<0.01$) were significantly different between the no fibrosis and moderate fibrosis groups. In comparison between these two groups, AST/platelet ratio with a cutoff point of 0.39; sensitivity of 67%, false positive error of 27%, positive predictive value (PPV) of 0.7 and negative predictive value (NPV) of 0.54, seemed to be a good tool for differentiating moderate fibrosis and no fibrosis (Fig 1).

In comparison between the severe fibrosis and no fibrosis groups, platelet/age ratio ($P<0.004$), AST/platelet ratio ($P< 0.002$), and platelet count

(P< 0.01) had statistically significant difference but only AST/platelet ratio with a cutoff point of 0.25, sensitivity of 76%, false positive error of 29%, PPV of 0.76, and NPV of 0.7 seemed an appropriate tool for differentiating between absence of fibrosis and severe fibrosis (Fig 2). Our results showed that between mild and moderate fibrosis groups, platelet count (P< 0.02) has a significant difference and it can differentiate between them with sensitivity of 76% and false positive error of 40% at the cutoff point of 158500 (Fig 3).

Table 1. The mean value of studied variables

Biochemical markers	No fibrosis	Mild fibrosis	Moderate fibrosis	Severe fibrosis
M:F ratio	20:6	31:14	26:5	17:11
Age (year)	35.27±6.31	35.47±13.01	36.10±12.72	43.85±16.70
Plt/age	6.84±3.40	6.06±2.94	5.44±5.75	4.19±2.80
AST/Plt	0.21±0.17	0.38±0.51	0.63±0.82	0.83±0.86
AST/ALT	0.84±0.44	1.03±0.80	1.10±0.74	1.84±2.33
Plt	211.40±87.82	189.15±58.72	149.32±74.06	153.61±73.11
ALT (U/L)	71.36±85.91	89.62±145.87	76.00±63.33	121.80±188.86
AST (U/L)	44.00±37.30	68.89±132.67	77.29±97.04	122.36±192.36
D Bill (mg/dl)	0.62±1.83	0.62±1.55	1.04±2.58	1.65±2.77
T Bill (mg/dl)	1.95±3.84	2.00±2.88	2.48±4.63	3.57±4.66
Alb (g/dl)	4.15±1.04	4.08±0.80	4.07±0.82	3.54±0.70
Chol (mg/dl)	168.86±39.01	158.52±43.10	184.00±48.55	146.07±30.13

M: male; F: female; Plt: platelet; AST: aspartate transferase; ALT: alanine transferase; D Bill: direct bilirubin; T Bill: total bilirubin; Alb: albumin; Chol: cholesterol

Data are presented as mean ± standard deviation.

Cholesterol levels were statistically different between the moderate and severe fibrosis groups (P<0.03). Age (P<0.02), platelet/age ratio (P<0.01), AST/platelet ratio (P<0.008), platelet count (P<0.02) and albumin levels (P<0.03) were significantly different between mild and severe fibrosis groups and from all these parameters, platelet count with a cutoff point of 151000, sensitivity of 83% and false positive error of 46% is a good tool for differentiating between the two groups (Fig 4). Albumin levels with a cutoff point of 3.6, sensitivity of 70% and false positive error of 36% seem to be an appropriate tool for differentiating mild and severe cases of fibrosis from each other (Fig 5). Serum bilirubin levels and AST/ALT ratio didn't have a significant difference between all groups of patients.

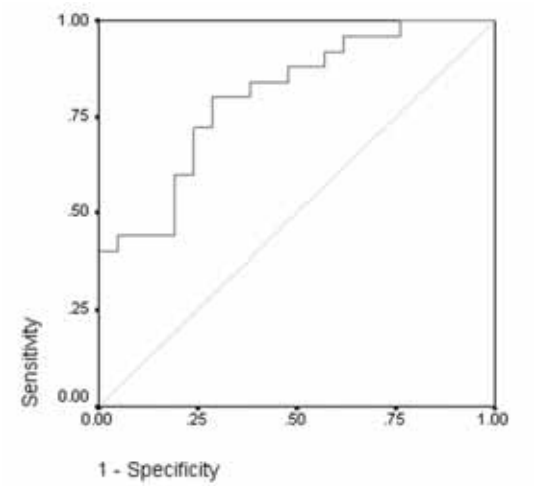

Figure 2. Roc curve for AST/Plt for differentiation of no fibrosis and severe fibrosis groups.

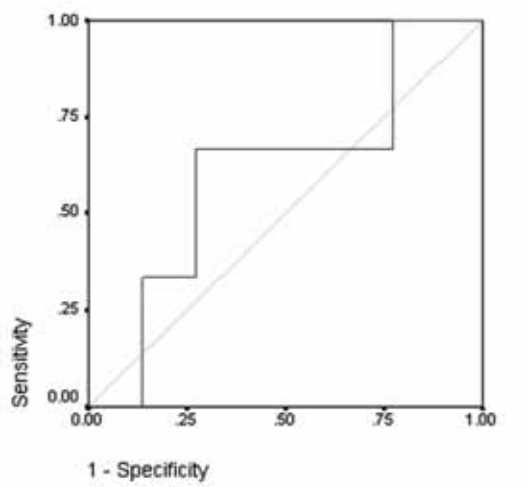

Figure 1. Roc curve for AST/Plt for differentiation of no fibrosis and moderate fibrosis groups.

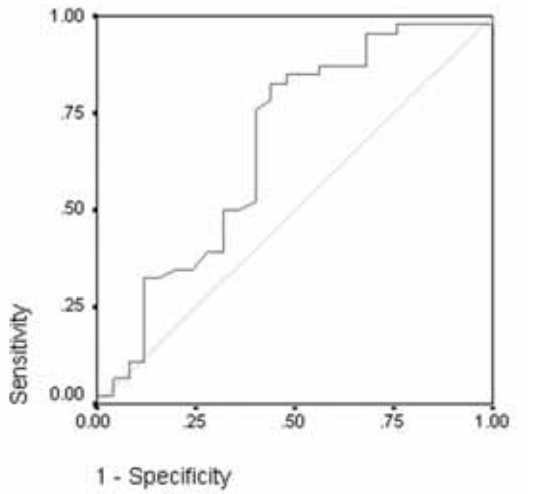

Figure 3. Roc curve for platelet for differentiation of mild fibrosis and moderate fibrosis groups.

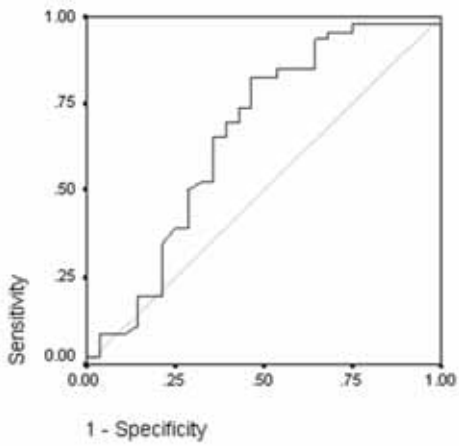

Figure 4. Roc curve for platelet for differentiation of mild fibrosis and severe fibrosis groups.

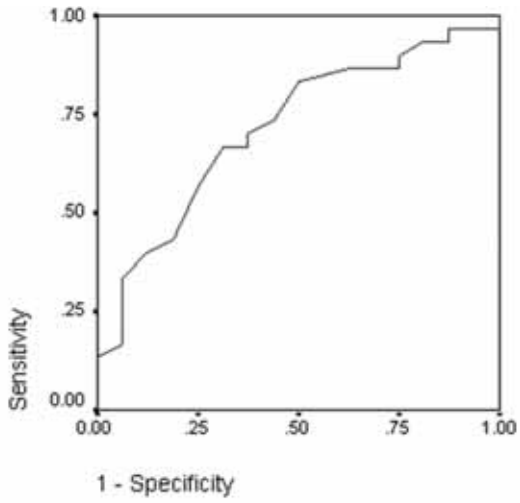

Figure 5. Roc curve for albumin for differentiation of mild fibrosis and severe fibrosis groups.

Discussion

The diagnosis of hepatic fibrosis had been relied on liver biopsy until now. It is invasive, costly and lacks adequate precision and accuracy which is why its use has been limited for this purpose and numerous studies has been conducted to find a noninvasive method of diagnosing this entity [18-20]. Since fibrosis is a direct result of imbalance between formation and destruction of extracellular matrix, it appears that measurements of the mentioned biomarkers may be useful in the diagnosis of fibrosis. In one study, the relationship between hepatic fibrosis and these markers was evaluated and it was finally recognized that increased amounts of extracellular proteins secondary to increased inflammatory activity lead to an increase in hepatic fibrosis [21].

Hyaloronate and collagen type 4 which precipitate in the peri-sinusoidal space following inflammation were introduced as specific markers of hepatic fibrosis by Masaki [22]. Measurements of these two markers are not routinely done in patients and not all laboratories are capable of measuring them. Their serum levels vary in different liver diseases and they are costly; so regardless of their sensitivity and specificity, they cannot be used as ideal markers for diagnosing hepatic fibrosis which is why some combination indexes like fibro-test and acti-test have limited usage.

Previous studies have reached cutoff points which only demonstrate the presence or absence of fibrosis [14]. In this study, patients with different degrees of fibrosis were compared with patients who didn't have fibrosis and cutoff points for AST/platelet ratio were determined to differentiate between patients with no fibrosis and moderate or severe degrees of fibrosis. We managed to find additional tools such as AST/platelet ratio, albumin levels and platelet counts for differentiating different levels of fibrosis and establishing cutoff points for each tool.

Platelet count had certain cutoff points for differentiating mild from moderate and mild from severe levels of fibrosis. Albumin also had suitable cutoff point for differentiating mild from severe fibrosis. Forns index is suitable for diagnosing mild cases of fibrosis but our study showed that platelet count, albumin levels, patient's age and AST/platelet ratio can be used to differentiate between mild and severe cases of fibrosis and significant differences of these markers exist between mild and severe forms of hepatic fibrosis. Lackner has reached the same results in his study [23].

White *et al.* reached a conclusion that in severe cases of hepatic fibrosis, levels of serum albumin increase [24] while Kelleher *et al.* study showed that in these patients, serum albumin levels decrease [25]. Our results are consistent with what Kelleher *et al.* found. Some studies suggest that AST/ALT ratio of greater than 1 is suggestive of cirrhosis. This tool has a low sensitivity [26, 27]. In Lackner *et al.* study, it was suggested that AST/platelet ratio is more accurate than AST/ALT ratio for diagnosing fibrosis [23]. This study suggests the same since AST/ALT ratio didn't have any significant difference between different groups of our patients while AST/platelet ratio did and it could be used to differentiate between moderate and severe cases of fibrosis.

As in previous studies [23, 28-30], we couldn't find a suitable tool for differentiating moderate from severe cases of fibrosis. Although serum cholesterol level had significant difference between these groups, a suitable cutoff point couldn't be established. Unlike many studies conducted until now [23, 26, 28, 31], our patient population wasn't only composed of hepatitis C

patients, and patients suffering from different reasons of hepatic fibrosis were included in the study and among them hepatitis C patients made the largest group (36.1%) while undiagnosed patients (26.1%) and HBV patients (23%) were placed second and third, respectively.

Level of hepatic fibrosis increases with age [17] and this fact was demonstrated in our study, as well. Unlike previous studies that introduced male gender as a factor that increases fibrosis [17], our data didn't show a significant difference in the level of fibrosis between men and women. Although numerous efforts have tried to find a suitable replacement for biopsy when it comes to diagnosing hepatic fibrosis and some had led to some degree of success, due to limitations that each test has, an ideal test for this purpose has not yet been introduced. It seems that a prospective study with a reference laboratory for measuring both routine markers and extracellular matrix components would be of great help in achieving this goal.

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