Gastroenterology and Hepatology From Bed to Bench. ***2010; 3(2): 71-76***

ORIGINAL ARTICLE

Prediction of liver histological lesions with biochemical markers in chronic hepatitis B patients in Iran

Seyed Moayed Alavian[1], Seyed Hoda Alavian[2], Neda Ashayeri[3], Marziyeh Babaei[2], Maryam Daneshbodi[2,] Bashir Hajibeigi[3]

[1] Baqiyatallah Research Center for Gastroenterology and Liver Disease, Baqiyatallah University of Medical sciences, Tehran, Iran

[2] General Practitioner, Researcher, Member of Tehran Hepatitis Center, Tehran, Iran

[3] General Practitioner, Iranian Blood Transfusion Organization Research Centre (IBTO), Tehran, Iran

ABSTRACT

Aim: In the current study, we aimed at identifying independent laboratory parameter to predict liver damage.

Background: Although liver biopsy is frequently recommended in assessing disease severity and selecting antiviral treatment candidates, it has several limitations.

Patients and methods: A total of 527 patients with untreated chronic hepatitis B virus infection were selected. A percutaneous liver biopsy was obtained. All of the patients were graded and staged for liver fibrosis (1-6) and inflammatory activity (1-18). Complete blood count, biochemical blood tests and Serum viral markers were detected for these patients.

Results: 106 patients had moderate to severe necroinflammatory activity. Compared to patients with mild activity, this group was older and had significantly higher aspartate aminotransferase (AST), alanine aminotrasferase (ALT), alkaline phosphatase (Alk. P) and AST to platelet ratio index (APRI) and significantly lowers hemoglobin (Hb), fasting blood sugar (FBS) and platelet (PLT). By multiple regressions analysis Hb, AST, FBS and PLT were independently predictive of moderate to severe necroinflammatory activity. 109 patients had moderate to severe fibrosis. Compared to patients with mild fibrosis, the former patients were older. In addition, the mean of AST, ALT, PT and APRI were significantly higher, and WBC, serum Alb and PLT were significantly lower in patients with moderate to severe fibrosis. Also by multiple regressions analysis age, AST, serum Alb and PLT were independently predictive of moderate to severe fibrosis.

Conclusion: Our study demonstrates the utility of biochemical markers for the diagnosis of moderate histological lesions in patients with chronic hepatitis B. Whereas millions of patients with chronic hepatitis B in the world are living, this approach will be useful and cost-benefit.

Keywords: *Chronic hepatitis B, Biomarker, Fibrosis, Necroinflammatory activity.*

(Gastroenterology and Hepatology From Bed to Bench 2010; 3(2): 71-76).

INTRODUCTION

The degree of hepatic necroinflammation (grade) and fibrosis (stage) are strongly associated with the natural history and risk of complications in patients with chronic hepatitis B (CHB), thus accurate diagnosis of liver fibrosis and cirrhosis is crucial to the management of patients with CHB (1-5).

Although liver biopsy is frequently recommended in assessing disease severity and

Received: 15 September 2009 *Accepted*: 25 January 2008
Reprint or Correspondence: Seyed-Moayed Alavian, MD. Baqiyatallah Research Center for Gastroenterology and Liver Diseases, Tehran, Iran
E-mail: editor@hepmon.com

selecting antiviral treatment candidates (4), it has several limitations: 1. This invasive procedure induces marked anxiety, abdominal or shoulder pain and discomfort. 2. It is well known that this method is associated with a significant rate of false negative results for the diagnosis of cirrhosis, particularly in the cases of macronodular cirrhosis. For example, the accuracy of the histological assessment of necroinflammation and fibrosis depends on size of the specimens (6-8). Furthermore, interpretation of the biopsies carries intraobserver and interobserver variation of 10-20% even among experienced pathologists (3, 6, 7, 9). 3. Side effects are observed frequently after liver biopsy and death may occur in some cases (10-12). 4. Another shortcoming of liver biopsy is its cost as it always requires hospitalization for 6-18 hrs (3, 13, 14). Considering these limitations and patient reluctance to undergo liver biopsy, noninvasive predictors of histology are desperately needed (1, 14).

Indices of biochemical markers demonstrated high predictive values for significant lesions in patients with chronic hepatitis C is developed (15-19), but there is not enough information regarding their use in HBV-infected patients. In the current study, we aimed at identifying independent clinical and laboratory parameter to predict liver damage. A regression model was derived based on these predictors.

PATIENTS and METHODS

This study was performed at Tehran hepatitis center (THC) between March 2004 and March 2006. A total of 527 patients with untreated chronic hepatitis B virus infection were prospectively and consecutively selected. CHB was defined by positive hepatitis B surface antigen (Hbs Ag) for at least 6 months and a compatible liver biopsy. Patients co-infected with HCV or HIV and patients with alcohol consume higher than 20 g/day alcohol for woman and 40 g/day for men were excluded.

A percutaneous liver biopsy was obtained from all patients. Samples were fixed with 10 % formaldehyde, embedded in paraffin and sliced, stained with hematoxylin-eosin, reticular fibers and collagen fibers. All of the samples were graded and staged for liver fibrosis (1-6) and inflammatory activity according to modified Kondell scoring (1-18).

Blood samples were collected after fasting for 8-12 h and at interval of no more than 3 months after biopsy. Red blood cells (RBC), white blood cells (WBC) hemoglobin (Hb) and platelet (PLT) were counted. Total serum bilirubin, triglyceride (TG), cholesterol (chol), prothrombin time (PT), gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) were determined. Based on these results and the AST) to platelet ratio index (APRI) index were calculated. The APRI index was established as follows: the AST levels the upper limit of normal (ULN)/ platelet counts (10^3/L) * 100 (20). Serum viral markers, including HBeAg and HBeAb were detected.

Analysis was carried out for all the data with SPSS 15. The primary outcomes were the detection of moderate to severe necroinflammatory activity (A7–A18) and moderate to severe necroinflammatory fibrosis (F5–F6). These thresholds were selected since they are generally considered indications for antiviral therapy. The independent discriminative values of the markers were determined using stepwise backward multiple logistic regression. A receiver–operator characteristic (ROC) curve was constructed to evaluate the discriminative power of each variable. A level of significance of 0.05 (5%) was adopted for all statistical analysis. All patients gave written informed consent to participate.

RESULTS

Five hundred and twenty-seven patients met the inclusion criteria. The mean of age (± standard deviation) was 33.56±12.36. 419 patients (79.5%) were male and 108 patients (20.5%) were female. We divided stages (fibrosis) into three groups: mild (F1-F2), moderate (F3-F4) and severe (F5-F6). Also we divided grades (necroinflammatory activity) into three groups: mild (A0-A6), moderate (A7-A12) and severe (A13-A18). Of the 527 patients 404 (78.8%) presented no or mild fibrosis, 84 (16.4%) had moderate fibrosis and 25 (4.9%) had severe fibrosis. The necroinflammatory activity of 421 (79.8%) patients was mild, of 99 (19.2%) patients was moderate and of 7 (1.4%) patients was severe.

106 patients (20.6%) had moderate to severe activity. Compared with patients with mild activity (n=409), this group had significantly higher AST ($p<0.0001$), ALT ($p<0.0001$), Alk.P ($p<0.02$) and APRI ($p<0.0001$) and significantly lower Hb ($p<0.03$), FBS ($p<0.04$) and PLT ($p<0.0001$). Patients with moderate to severe necroinflammatory activity had a significantly higher prevalence of positive HBe-Ag ($p<0.04$). According to our result there were not any correlation between necroinflammation activity and cholesterol, bilirubin, WBC, Alb, PT and TG (Not significant: NS). By multiple regressions analysis Hb, AST, FBS and PLT were independently predictive of moderate to severe necroinflammatory activity. Tables 1 and 2 present the area under the curve and sensitivity and specificity of each marker used for the diagnosis of moderate to severe necroinflammatory activity.

109 patients (21.3%) had moderate to severe fibrosis. Compared with patients with mild fibrosis (n=404), the former patients were older ($p=0.001$). In addition, the mean of AST ($p<0.0001$), ALT ($p<0.0001$), PT ($p<0.02$) and APRI ($p<0.0001$) were significantly higher, and WBC ($p<0.0001$), serum Alb ($p<0.0001$) and PLT ($p<0.0001$) were significantly lower in patients with moderate to severe fibrosis.

Table 1. Sensitivity, specificity and area under the curve of the noninvasive markers studied for the diagnosis of moderate to severe necroinflammation of liver.

	Cut-off	AUC*	Sensitivity (%)	Specificity (%)
AST†	45.5	0.668	73	56
APRI‡	1.7	0.709	82	41
ALT§	58	0.614	65	47
Alk.P‖	155.5	0.559	67	41

* Area Under the Curve; † Aspartate aminotransferase; ‡ Aspartate aminotransferase to platelet ratio index; § Alanine aminotransferase; ‖ Alkaline phosphatase

Our statistical results did not show any association between fibrosis and Hb, FBS, Alk.P, bilirubin, cholesterol and TG (NS).

Table 2. Biochemical markers associated with moderate to severe fibrosis according to multiple logistic regression analysis.

	Cut-off	AUC*	Sensitivity (%)	Specificity (%)
ALT†	54.5	0.615	71	42
AST‡	43.5	0.648	72	53
PLT§	129500	0.534	78	23
APRI‖	1.7	0.706	80	41

* Area Under the Curve; † Alanine aminotransferase; ‡ Aspartate aminotransferase; § Platelet; ‖ Aspartate aminotransferase to platelet ratio index

By multiple regressions analysis age, AST, serum Alb and PLT were predictive of moderate to severe fibrosis.

DISCUSSION

Liver biopsy has limitations, including sampling artifact and inter-individual as well as intra-individual variability in scoring. Furthermore, serial liver biopsies are not a practical means of assessing fibrosis progression due to potential complications, costs, and patient/physician reluctance (21, 22). Although liver biopsy has these limitations, it has remained

the "gold standard" mainly because of the absence of better alternatives.

In the last decade, many studies have been dedicated to the search of non invasive markers able to provide accurate information about liver fibrosis and necrosis in patients with chronic, potentially progressive, hepatic diseases.

In HCV-infected patients, the biomarkers are accurate and highly reproducible (23). We assessed these indices in patients with chronic hepatitis B. Millions of patients with CHB in the world are living and if we find the severity of disease without performing the liver biopsy, it will be cost-benefit for approach.

Our results illustrate their potential usefulness, in predicting the severity of necroinflammation and fibrosis. In our research, Histological activity was associated with AST and ALT levels. The same finding has been reported in previous studies (1, 24, 25). As previously reported, in our study with the inflammation becoming serious, PLT tended to decrease (24, 25). Also we found a relationship between necroinflammation and Alk.P that was similar to previous research (25). Other serum markers that had correlation with grade of inflammation were Hb and FBS. To our knowledge, this is the first study to examine these markers in this population.

On the contrary with our study, Lun showed that viral replication parameters such as HBeAg have no correlation with severity of necroinflammation and fibrosis (24). In our research, Fibrosis had association with AST and ALT. The same results were reported in previous studies (1, 6). Also as previously reported in patients with hepatitis C, we found relationship between fibrosis and APRI in our population (3, 22, 26, 28). In accordance with previous reports, the patients with higher degree of fibrosis were older (28) and had higher PT (8, 29). In our research, fibrosis had association with Alb and PLT that was similar to results of previous study on patients with hepatitis C (25). Also we found relationship between fibrosis and WBC that to our knowledge there was not any study to evaluate this marker in hepatic fibrosis.

By the way, multiple regressions analysis showed that age, high levels of AST, and low levels of Alb and PLT were independently predictive of moderate to severe fibrosis. It will be better to assume that AST elevation is more important than ALT elevation in predication of fibrosis. Independent factors associated with more severity in grade and stage in our study will guide us to consider these factors in earlier decision for treatment.

Our study has several limitations. It was cross-sectional; the responsiveness of these indices to spontaneous or treatment-induced histological changes remains unclear. Second, the majority of our patients were HBeAg-negative; these results may not be applicable to institutions where this profile is less common.

The greater accuracies of biomarkers, when assessed with biopsy specimens greater than 15 mm versus smaller biopsies, suggest that some discordance between biomarkers and histology that were due to biopsy specimen sampling error[30]. Several case reports have observed false negatives of liver biopsy versus biochemical markers (30).

Our study demonstrates the utility of biochemical markers for the diagnosis of moderate to severe histological lesions in patients with chronic hepatitis B.

REFERENCES

1. Myers RP, Tainurier MA, RatziuVL, Piton AN, Piton A, Thibault V, et al. Prediction of liver histological lesions with biochemical markers in patients with chronic hepatitis B. Hepatology 2003; 39: 222-30.

2. Lee WM. Hepatitis B virus infection. N Engl J Med 1997; 337: 1733-45.

3. Sebastiani GI, Alberti AL. Non-invasive fibrosis biomarkers reduce but not substitute the need for liver biopsy. World J Gastroenterol 2006; 12: 3682-94.

4. Lok ASF, Mcmahon B. AASLD practice guidelines: chronic hepatitis B. Hepatology 2004; 39: 857-61.

5. Rockey DO, Bissell MO. Non-invasive measures of liver fibrosis. Hepatology 2006; 43: s113-s20.

6. Hui AY, Chan HL, Wong VW, Liew CT, Chim AM, Chan FK, et al. Identification of chronic hepatitis B patients without significant liver fibrosis by a simple non-invasive prediction model. Am J Gastroenterol 2005; 100: 616-23.

7. Poon TC, Hui AY, Chan HL, Ang IL, Chow SM, Wong N, et al. Prediction of liver fibrosis and cirrhosis in chronic hepatitis B infection by serum proteomic fingerprinting: a pilot study. Clin Chem 2005; 51: 328-35.

8. Colloredo G, Guido M, Sonzogni A, Leandro G. Impact of liver biopsy size on histological evaluation of chronic viral hepatitis: the small sample, the milder the disease. J Hepatol 2003; 39: 239-44.

9. Hubscher SG. Histological grading and staging in chronic hepatitis: clinical applications and problems. J Hepatol 1998; 29: 1015-22.

10. Gunneson TJ, Menon KV, Weisner RH, Daniels JA, Hay JE, Charlton MR, et al. Ultrasound-assisted percutaneous liver biopsy performed by a physician assistant. Am J Gastroenterol 2002; 97: 1472-75.

11. Cadranel JF, Rufat P, Degos F. Practices of liver biopsy in France: result of a prospective nationwide survey for the group of epidemiology of the French association for the study of the liver (AFEF). Hepatology 2000; 32: 477-81.

12. Poynard T, Ratziu V, Bedossa P. Appropriation of liver biopsy. Can J Gastroenterol 2000; 14: 543-48.

13. Wong JB, Koff RS. Watchful waiting with periodic liver biopsy versus immediate empirical therapy for histologically mild chronic hepatitis C. A cost-effectiveness analysis. Ann Intern Med 2000; 133: 665-75.

14. Fredman SL, Maher JJ, Bissel DM. Mechanisms and therapy of hepatitis fibrosis: report of the AASLD single topic basic research conference. Hepatology 2000; 32: 1403-408.

15. Imbert-Bismut FR, Ratziu V, Pieroni L, Charlotte F, Benhamou Y, Poynard T; MULTIVIRC Group. Biochemical markers of liver fibrosis in patient with HCV infection: a prospective study. Lancet 2001; 357: 1069-75.

16. Myers RP, Benhamou Y, Imbert-Bismut F, Thibault V, Bochet M, Charlotte F, et al. Serum biochemical markers accurately predict liver fibrosis in HIV and hepatitis C virus- coinfected patients. AIDS 2003; 17: 721-25.

17. Myers RP, Messous D, Thabut D, Imbert-Bismut F, Ratziu V, Mercadier A, et al. The prediction of fibrosis with serum biochemical markers in patients with chronic hepatitis C: prospective validation in 534 patients (abstract). Hepatology 2002; 36: 351A.

18. Poynard T, Munteanu M, Imbert-Bismut F, Charlotte F, Thabut D, Le Calvez S, et al. Prospective analysis of discordant results between biochemical markers and biopsy in patients with chronic hepatitis C. Clin Chem 2004; 50: 1344-55.

19. Gebo KA, Herlong HF, Torbenson MS, Jenckes MW, Chander G, Ghanem KG, et al. Role of liver biopsy in management of chronic hepatitis C. A systemic review. Hepatology 2002; 36: s161-s72.

20. Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology 2003; 38: 518–26.

21. Thuluvath P, Krok K. Noninvasive markers of fibrosis for longitudinal assessment of fibrosis in chronic liver disease. Am J Gastroenterol 2005; 100: 1981-83.

22. Parise ER, Oliveira AC, Figueiredo-Mendes C, Lanzoni V, Martins J, Nader H, et al. Noninvasive serum markers in the diagnosis of structural liver damage in chronic hepatitis C virus infection. Liver Int 2006; 26: 1095–99.

23. Poynard T, McHutchison J, Manns M, Myers RP, Albrecht J. Biochemical markers as surrogate markers of liver fibrosis and activity in patients infected by hepatitis C virus: an example in a randomized trial of pegylated interferon alfa-2b and ribavirin combination. Hepatology 2002; 36: 351A.

24. Lu L, Zeng M, Mao Y, Li J, Qiu D, Fang J, et al. Relationship between clinical and pathologic findings in patients with chronic liver diseases. World J Gastroenterol 2003; 9: 2798-800.

25. Lu L, Zeng M, Wan M, Li C, Mao Y, Li J, et al. Grading and staging of hepatic fibrosis, and its relationship with noninvasive diagnostic parameters. World J Gastroenterol 2003; 9: 2574-78.

26. Lackner C, Struber G, Liegl B, Leibl S, Ofner P, Bankuti C, et al. Comparison and validation of simple noninvasive tests for prediction of fibrosis in chronic hepatitis C. Hepatology 2005; 41: 1376-82.

27. Giannini E, Risso D, Botta F, Chiarbonella B, Fasoli A, Malfatti F, et al. Validity and clinical utility

of the aspartate aminotransferase-alanine aminotransferase ratio in assessing disease severity and prognosis in patients with hepatitis C virus-related chronic liver disease. Arch Intern Med 2003; 163: 218-24.

28. Forns X, Ampurdans S, Liovet JM, Aponte J, Quintó L, Martínez-Bauer E, et al. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. Hepatology 2002; 36: 986-92.

29. Croquet V, Vuillemin E, Ternisien C, Pilette C, Oberti F, Gallois Y, et al. Prothrombin index is an indirect marker of severe liver fibrosis. Eur J Gastroenterol Hepatol 2002; 14: 1133-41.

30. Poynard T, McHutchison J, Manns M, Myers RP, Albrecht J. Biochemical surrogate markers of liver fibrosis and activity in a randomized trial of peginterferon alfa-2b and ribavirin. Hepatology 2003; 38: 481-92.