Non-invasive parameters in the assessment of liver fibrosis.

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Abstract

Liver biopsy and histopathological evaluation are the main methods to assess liver pathology. However, liver biopsy is not widely used due to implementation challenge. We aimed to investigate non-invasive parameters for evaluation of liver fibrosis in chronic Hepatitis B patients. A total of 65 patients who admitted to outpatient clinic and diagnosed as chronic hepatitis were included in this study. Liver puncture biopsy was performed for all patients and was evaluated according to the Modified Ishak Fibrosis Score. The patients without fibrosis or with mild and moderate fibrosis were evaluated as Group I (Stage O, I, II), the patients with advanced fibrosis were evaluated as Group II (stage III, IV, V, VI). Among 65 patients, 42 were male and 23 were female; 48 of the patients were in group I while 17 patients belonged to group II. Gamma-Glutamyl Transferase (GGT), Alkaline Phosphatase (ALP), Aspartat Transaminaz (AST) and Alanin Aminotransferaz (ALT) levels were significantly higher in Group II patients. In the assessment of liver fibrosis, liver biopsy remains the gold standard diagnostic method but AST, ALT, GGT, ALP parameters have contribution to this evaluation. Non-invasive testing may be useful in cases which biopsy cannot be performed or repeat biopsy required.

Introduction

Cirrhosis of the liver is a common disease that goes with fatal complications [1-3]. Depending on age and other factors, 5-10% of hepatitis B infections, 60-80% of hepatitis C infections and 80-90% of hepatitis D infections become chronic [4-6]. In most patients, symptoms are not observed until moderate or severe fibrosis developed in liver tissue. Many of the patients are diagnosed after development of portal hypertension, ascites, oesophageal variceal haemorrhage and splenomegaly. Rapid diagnostic methods are needed for it [7]. Bilirubin, Aspartat Aminotransferaz (AST), Alanin Aminotransferaz (ALT), albumin, gamma globulin, alpha 2 macroglobulin, haptoglobulin, apolipoprotein A1 serum levels, platelet count, prothrombin time and activity may reflect liver fibrosis and liver reserves [8]. By using these parameters, the new sensitive indexes-Bonacini, Forns index and APRI, ALT/AST ratio, FibroTest and Actitest-have been developed [9-12].

We aimed to investigate the importance of non-invasive parameters in evaluation of liver fibrosis and determine the relationship with fibrosis in chronic Hepatitis B patients.

Materials and Methods

Study design and patient selection

This study was conducted in Dicle University Medical Faculty, Department of Infectious Diseases and Clinical Microbiology. A total of 65 patients who admitted to outpatient clinic and diagnosed as chronic hepatitis were included in this study. All of the patients have had high levels of liver enzymes for at least six months and percutaneous liver biopsy was performed for histopathological diagnosis. Liver biopsies of patients were evaluated according to the modified Ishak fibrosis score. Two groups of patients were formed. According to the degree of fibrosis stages, Stages 0-II were considered as Group I (no fibrosis, mild or moderate fibrosis), Stages III-VI were

considered as Group II (advanced or severe fibrosis). The following laboratory investigations were tested for the patients: Blood biochemistry: AST, ALT, Gamma-glutamyl transferase (GGT), Alkaline Phosphatase (ALP), bilirubin, albumin, gamma globulin, Alpha-Fetoprotein (AFP). Complete Blood Count (WBC): haemoglobin, haematocrit, platelets, Mean Corpuscular Volume (MCV) Hepatitis B virus surface antigen (HBsAg), hepatitis B virus e antigen (HBeAg), Hepatitis B Virus DNA (HBV DNA) and prothrombin time and activity. In both groups, demographic and laboratory values were statistically compared Biochemical analyses (AST, ALT, GGT, bilirubin days) were performed in Dicle University Faculty of Medicine Laboratories by Abbott Architect C 8000-Abbott System. Alpha fetoprotein was measured by BioDPC of Immulite 2000-US devices, blood counts were measured by the Cell-DYN 3700-Abbott. Prothrombin activity was determined using STA-Compact Diagnostica Stago coagulometer device. Viral serologic parameters were tested using Vitros ECI and immunodiagnostic system and antiHBe AXSYM system, Abbott based on ELISA method. HBV DNA was tested by the COBAS® AmpliPrep/COBAS® TaqMan® HBV Test, v 2.0 by Roche molecular systems. Liver biopsy samples were sent to Pathology Laboratory. Samples were evaluated in terms of activity with haematoxylin-eosin stain while in terms of fibrosis "Masson's trichrome" stain.

Exclusion criteria

The patients with viral hepatitis other than Hepatitis B, chronic liver diseases such as toxic hepatitis, granulomatous hepatitis, autoimmune hepatitis, cholestatic liver disease, alcoholic liver disease, Wilson's disease, alpha-1 antitrypsin deficiency and hemochromatosis, primary or metastatic liver cancer; systemic diseases such as nephropathy and congestive heart failure were excluded from the study. The study, only patient files were retrospectively evaluated. Thus, we could not get ethical approval.

Statistical review

Statistical evaluation of the data was performed using SPSS software. Statistical analysis of the continuous variables between two groups was compared by "Mann Whitney" test. In the comparison of categorical values for the groups, chi-square test; in appropriate cases "Fisher's exact test" was used. Descriptive statistics were presented as mean ± Standard Deviation (SD). All tests were evaluated "two-tailed" and p<0.05 was interpreted as significant.

Results

Among 65 patients included in the study, 42 were male and 23 were female. The patients without fibrosis or patients with mild and moderate fibrosis were evaluated as Group I (Stages 0-II), while the patients with advanced fibrosis were evaluated as Group II (stages III-VI).

Comparison of general features of cases

In the study, 47 of the patients were in group I while 18 patients belonged to group II. Of Group I patients 18 (38.2%) were female, 29 (61.7%) were male. Among Group II cases 13 (72.2%) were male and the rest were female. In terms of gender, there was not a significant difference in both groups. Mean age of Group I patients was 31 ± 11.96 and mean of Group II was 29. ± 12.19. Both groups had no significant difference when compared in terms of age ALT was 73.42 ± 111.03 in Group I and 97.89 ± 86.50 in Group II. AST and ALT values between Groups I and II were significantly different and both parameters were higher in Group II patients. In Group I patients, AST/ALT ratio was 0.79 ± 0.30 and in Group II patients this ratio was 0.66 ± 0.16. Group I and Group II patients' ratio did not differ significantly with AST/ALT. Both groups were not significantly different when compared in terms of platelet count and viral load. Total bilirubin (mg/dl) and prothrombin time was not a significant difference between the groups. In terms of albumin, gamma-globulin (g/dl) and albumin/globulin ratio, there was no significant difference in both groups mean AFP level (ng/ml) was 1.90 ± 0.98 in Group I patients, and 4.31 ± 5.29 in Group II; a significant difference in both groups was not found (Table 1).

Table 1. The clinical and demographic data of the study groups.

	Group 1 (N=48%)	Group 2 (N=17%)	P
Mean age	31 ± 11.96	29 ± 12.19	0.644
Gender (M/F)	29/18	13-May	0.427
AFP	1.99 ± 0.98	4.31 ± 5.29	0.316
AST	43.51 ± 56.83	57.28 ± 37.36	0.006
ALT	73.42 ± 111.03	97.89 ± 86.50	0.009
T. bil	0.73 ± 0.35	0.75 ± 0.45	0.778
INR	1 ± 0.7	0.98 ± 0.21	0.030
Albumin	1.26 ± 0.25	1.12 ± 0.15	0.030
PLT	231.41 ± 54.49	215.33 ± 44.27	0.343
Globulin	3.37 ± 0.42	3.64 ± 0.31	0.030
AST/ALT	0.79 ± 0.30	0.66 ± 0.16	0.162
HBV DNA	40280.45 ± 71041.01	58017.10 ± 81511.18	0.073
PTZ	12.18 ± 0 .85	12.45 ± 0.82	0.325

Discussion

By viral serology and PCR (polymerase chain reaction), chronic liver disease is determined in asymptomatic patients. With recently developed immunomodulatory and antiviral drugs, treatment of the disease before the development of cirrhosis is possible. Response to treatment is more effective in cases detected in early stages. In addition to biochemical and serological diagnostic methods, liver biopsy and histopathological evaluation were the main methods to assess

liver pathology since nearly one hundred years [13,14]. However, liver biopsy is not widely used due to implementation challenge, because it is an invasive method and cause complications [15,16]. The need for repeats of biopsy in staging of disease and in detecting of response to treatment and implementation challenge due to the presence of ascites and coagulopathy in patients with advanced disease are disadvantages [17-20]. Our study was important due to investigate biochemical and other non-invasive diagnostic methods could replace the histopathology and detect compatibility of these methods with histopathology.

In our study, the big majority of the patients were male and when we compared the two groups in terms of age and gender, significance was not found between two groups. In a Korea study, 188 chronic hepatitis B patients were followed for 119.8 months, demographic characteristics were evaluated; female/male ratio was 3.9/1 and the mean age was found to be 35, over 40 years based on time (10-15 years) increased risk is indicated in cirrhosis development. This height is explained by increased risk of cirrhosis in later life and the longer duration of the disease [21]. Similarly, in a multicentric study conducted in China with 200 cases, is reported to increase fibrosis with age [22]. In our cases, a significant correlation (p=0.104) was not found between duration of disease and the degree of fibrosis. These results can be explained by regional differences, and with viral mutation types, and the small number of cases. In a few studies, relationship between fibrosis and HBeAg was not detected and necroinflammation was stated to be in relation with fibrosis [7,21,23]. Due to the small number of cases and most of them being HBeAg-negative; correlation analyse could not be investigated by the presence of HBeAg between the biochemical parameters and the degree of fibrosis. In studies conducted in patients with chronic hepatitis, relationship between viral load (HBV DNA, HCV RNA) and fibrosis development were also discussed. In our study, there was no significant difference between advanced degree of fibrosis and viral load (p=0.07). Similarly, Lu et al. [22] did not find a correlation between viral load and inflammatory activity among 200 viral hepatitis patients. However, Mohamadnejad et al. [7] detected a correlation between viral load and fibrosis in 276 viral hepatitis B patients.

Transaminases are most significant predictor of necroinflammatory activity in acute and chronic liver disease [21,24]. However, after the development of cirrhosis, even in the presence of severe necroinflammation, due to reduced hepatocyte mass, high levels of transaminase levels should not be anticipated. In general, necroinflammation at what rate can result in fibrosis in acute exacerbations of hepatitis is unpredictable. Therefore, just transaminase levels are not available to assess fibrosis [21,25,26]. Indeed Wong et al. [4], study of 130 HCV patients, ALT and AST activity and fibrosis scores were not compatible with necroinflammatuar activity. However Park et al. [21] stated that significant and continuous transaminase accelerates the development of cirrhosis. In our study, between ALT, AST levels at the time of biopsy and the degree of fibrosis, significance was found (p<0.05). In recent studies it was reported that in patients with chronic viral hepatitis, AST/ALT ratio was more meaningful in showing fibrosis than alone AST or ALT [8,24] was. Giannini et al. [6] including 252 HCV patients, retrospective study; it was reported that AST/ALT ratio indicated cirrhosis with 78% sensitivity and 97% specificity, also it was stated that if AST/ALT ratio was considered together with thrombocytopenia (<130000/mm^3), the positive predictive value was 97%, negative predictive value was 86%. Furthermore, in 63 cirrhotic patients who were followed for a year, AST/ALT ratio>1.6 was stated to be equivalent to Child-Pugh and MELD scores in predicting prognosis [6]. In chronic viral hepatitis as well as non-alcoholic steatohepatitis, the AST/ALT ratio>1 and thrombocytopenia are reported to exhibit severity of fibrosis. In a Turkey study Aydin et al. [27] were also found that a low platelet count and AST/ALT ≥ 1 are highly compatible with fibrosis in 140 viral hepatitis patients with chronic liver disease. Lacobellis et al. [28] also reported that low platelet levels (<140.000/mm^3) have higher sensitivity for evaluating in cirrhosis in patients with chronic. In our study, AST/ALT ratio was not a significant marker in distinguishing groups with and without severe fibrosis. In our study, AST/ALT ratio in both groups mean<1'idi. To explain this issue, further studies involving a large number of cases with long- term follow-up is considered to be beneficial. In our study and Arhan et al. study, significance was not found in platelet levels between patients with no or mild fibrosis and patients with advanced fibrosis. These unexpected results may be due to small number of cases, laboratory error, yet no development of portal hypertension and clinical decompensation. Also scattered settlements of fibrosis in the liver are considered to affect the results. Classically, decreased prothrombin activity, prolonged prothrombin time, increased INR and gamma-globulin were seen in chronic hepatitis. After the development of cirrhosis (advanced stage of fibrosis) as well as these pathological laboratory findings, hypoalbuminemia emerges. The low number of platelets is an important finding indicating chronic hepatitis cirrhosis. Clinical status of patients, histopathology, AST, ALT, AST/ALT ratio, ALP, GGT, AFP, level of bilirubin were assessed [10,11,27,28]. When the study groups in terms of INR and prothrombin activity, statistically significant differences were not found between the groups. In Chronic liver disease due to necroinflammation and fibrosis, levels of albumin decreased and gamma-globulin is increased [29]. In our study, albumin, gamma-globulin and, albumin/globulin ratio was not significantly different between both groups. In general, the height of bilirubin in acute and chronic hepatitis is known to be not associated with the prognosis. In advanced cirrhosis, hyperbilirubinemia may suggest a poor prognosis [10]. Relationship between necroinflammation and level of bilirubin cannot be explained. In 235 chronic hepatitis B patients, Hui et al. [10] found that hyperbilirubinemia, hypoalbuminemia, and thrombocytopenia have been associated with increased fibrosis.

The alpha feto protein values also increases in necroinflammation. In the few studies, high AFP levels were suggested to be associated with fibrosis although it is unclear

[10,19]. In our study, there was no significant difference in terms of AFP and total bilirubin levels between the groups. These results suggested that due to the limited number of cases.

Some studies reported ALP and GGT elevations were found to be related with necroinflammation and fibrosis in the liver [30]. In our study, these parameters were significantly higher in Group II patients. The sensitivity of multiple test parameters in demonstrating fibrosis was found in a wide range (17-80%) and liver biopsy was reported to be the basic diagnostic method in chronic hepatitis [31].

A major limitation to our study is the relatively small sample size. Our patient population was not large enough to reach a definite result. There is a need for studies conducted on a larger patient group.

As a result, in the assessment of liver fibrosis, liver biopsy remains the gold standard diagnostic method. In our study, we saw that AST, ALT, GGT, ALP parameters have contribution to this evaluation. In cases of biopsy cannot be performed and requiring repeat biopsy, non-invasive testing may be useful.

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