Predictors of Liver Fibrosis in Patients with Chronic HCV Infection

Kronik HCV Enfeksiyonlu Olgularda Karaciğer Fibrozunun Derecesini Belirleyen Değişkenler

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Geliş Tarihi/*Received:* 21.07.2011
Kabul Tarihi/*Accepted:* 30.05.2012

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ABSTRACT Objective: The aim of this study was to investigate the independent variables for the degree of liver fibrosis in patients with chronic hepatitis C virus (HCV) infection. **Material and Methods:** In this cross-sectional retrospective study, the patients were divided in two groups as Group A (patients with fibrosis score 0-2) and Group B (patients with fibrosis score 3-4) and they were compared in terms of age, gender, body mass index, alcohol consumption, presence of diabetes, serum HCV RNA, alanine aminotransferase, gamma-glutamyl transferase, and aspartate aminotransferase, total bilirubin, albumin, globulin and alkaline phosphatase levels, prothrombin time, blood thrombocyte count, splenomegaly in ultrasonographic examination, HCV genotype and liver steatosis. **Results:** Of 201 patients, 93 (46.3%) were males; the median age was 51 (range: 18-77) years. It was found that serum gamma-glutamyl transferase and aspartate aminotransferase levels, age, prothrombin time and the presence of splenomegaly were independent variables predictive of advanced liver fibrosis. Odds ratio and p values were 1.984 and 0.013 for gamma-glutamyl transferase, 1.633 and 0.018 for aspartate aminotransferase, 1.861 and 0.003 for age, 5.598 and 0.008 for splenomegaly and 0.541 and 0.007 for prothrombin time, respectively. **Conclusion:** The results of this study suggested that advanced age, elevated serum gamma-glutamyl transferase and, aspartate aminotransferase levels, prolonged prothrombin time and the presence of splenomegaly are independent variables determining severe liver fibrosis.

Key Words: Liver cirrhosis; hepacivirus

ÖZET Amaç: Bu çalışmanın amacı kronik hepatit C virüsü (HCV) enfeksiyonlu olgularda karaciğer fibrozunun derecesini belirleyen bağımsız değişkenleri araştırmaktı. **Gereç ve Yöntemler:** Bu geriye dönük kesitsel çalışmada hastalar Grup A (fibroz skoru 0-2 olanlar) ve Grup B (fibroz skoru 3-4 olanlar) olarak iki gruba ayrıldı ve gruplar yaş, cinsiyet, beden kitle indeksi, alkol kullanımı, diyabet varlığı, serum HCV RNA, alanin aminotransferaz, gama-glutamil transferaz, aspartat aminotransferaz, total bilirubin, albumin, globulin ve alkalen fosfataz seviyeleri, protrombin zamanı, kan trombosit sayısı, ultrasonografide splenomegali varlığı, HCV genotip ve karaciğer yağlanması açısından karşılaştırıldı. **Bulgular:** İkiyüz bir hastadan 93 (%46,3)'ü erkekti ve ortanca yaş 51 (alt ve üst sınırlar: 18-77 yıl) idi. Serum gama-glutamil transferaz ve aspartat aminotransferaz düzeyleri, yaş, protrombin zamanı ve splenomegalinin ileri karaciğer fibrozunu belirleyen bağımsız değişkenler olduğu bulundu. Odds oranı ve p değerleri gamaglutamil transferaz için 1,984 ve 0,013; aspartat aminotransferaz için 1,633 ve 0,018; yaş için 1,861 ve 0,003; splenomegali için 5,598 ve 0,008 ve protrombin zamanı için 0,541 ve 0,007 idi. **Sonuç:** Bu çalışmanın sonuçları bize ileri yaş, yüksek serum gamaglutamil transferaz ve aspartat aminotransferaz düzeyleri, uzamış protrombin zamanı ve splenomegali varlığının ileri karaciğer fibrozunu belirleyen bağımsız değişkenler olduğunu olduğunu düşündürmüştür.

Anahtar Kelimeler: Karaciğer sirozu; hepasivirüsler

doi: 10.5336/medsci.2011-25802

Turkiye Klinikleri J Med Sci 2012;32(6):1522-9

Chronic hepatitis C virus (HCV) infection has an unpredictable clinical course, and some patients may develop serious complications such as liver cirrhosis or hepatocellular carcinoma.[1] Combination therapy of chronic HCV infection with pegylated interferon and ribavirin achieves 55-60% sustained virologic response.[2] However, the use of this costly treatment with serious side effects is controversial in patients with a low probability for development of advanced liver fibrosis. In cases where liver biopsy is not an option, variables predictive of liver fibrosis progression become essential in treatment decisions.[3-23] The predictors of liver fibrosis were reported as long duration of chronic HCV infection, advanced age, alcohol use, elevated serum alanine aminotransferase (ALT) levels, high body mass index (BMI) and fatty infiltration or elevated histologic activity index in liver biopsy.[3-23] However; when we analyzed these studies, we realized that only some variables that might predict liver fibrosis were evaluated in every study, and studies including most of the variables were only a few.In our study; different from other studies, we have evaluated a number of variables which are commonly used in clinical practice and may predict fibrosis score, and tried to find out the independent variables that determined the liver fibrosis. The aim of this study was to investigate the independent variables for the degree of liver fibrosis in Turkish patients with chronic HCV infection.

MATERIAL AND METHODS

Medical files of 201 patients treated for chronic HCV infection in our Chronic Hepatitis Outpatient Clinic between January 2001 and January 2010 were retrospectively analyzed. The inclusion criteria were diagnosis of chronic HCV infection, liver biopsy assessed using the modified Knodell scoring system and being treatment-naïve for HCV infection before the biopsy.

Patients with autoimmune hepatitis, hemochromatosis, acute viral hepatitis, malignancy, pregnancy, co-infection with HBV or HIV, with former treatment for chronic HCV infection before tests or biopsy were excluded.

Patients' sociodemographic and medical data including age, gender, height, weight, alcohol consumption, presence of diabetes, serum HCV RNA, ALT, gamma-glutamyl transferase (GGT), and aspartate aminotransferase (AST), total bilirubin, albumin, globulin and alkaline phosphatase (ALP) levels, prothrombin time, blood thrombocyte count, splenomegaly in ultrasonographic examination, HCV genotype, steatosis, histopathologic activity index by the modified Knodell scoring system and fibrosis score were retrieved from their medical records.

The patients were grouped in two groups as Group A (patients with fibrosis score 0-2) and Group B (patients with fibrosis score 3-4) and compared.

HCV RNA QUANTIFICATION

HCV-RNA quantification was done using branched DNA signal amplification (Versant HCV RNA 3.0 Assay, Bayer Corporation Diagnostics, USA; detection range 615-7690000 U/mL) or real-time polymerase chain reaction (Cobas TaqMan HCV test v 2.0; detection range 25-391000000 U/mL). HCV genotype was assessed by Innolipa HCV II (Bayer Diagnostics, USA). We divided the cases in two groups as HCV RNA level ≥800.000 U/mL and <800.000 U/mL, considering the effect of HCV RNA level fluctuations.

ASSESSMENT OF LIVER HISTOLOGY

Liver biopsy was assessed using the modified Knodell scoring system.

STATISTICAL ANALYSIS

Data were analyzed using SPSS-13 (SPSS Inc., Chicago, IL). Continuous variables were expressed as median and minimum-maximum measurements and categorical variables were expressed as number and percentage of patients. The patients with mild and severe fibrosis scores were compared using the χ^2 test for categorical variables and the Mann Whitney-U test for continuous variables without normal distribution. The logistic regression analysis was used for the multiple regression analysis of independent factors affecting fibrosis

score. When we performed logistic regression analysis including prothrombin time, GGT, AST and age values, we used the rates of this values to standard deviations. The level of significance was set as $p<0.05$.

The approval of ethics committee was not obtained because of the retrospective nature of the study.

RESULTS

Of patients, 93 (46.3%) were males; the mean age was 51 (range: 18-77) years (Table 1). Comparative data for patient groups with mild and severe fibrosis are summarized in Table 2. The age (p=0.001), prothrombin time (p=0.001), serum AST (p=0.001), globulin (p=0.017), ALP (p=0.019), ALT (p=0.001) and serum GGT (p=0.001) levels were higher in the group with a severe fibrosis score.

Serum albumin level and blood thrombocyte counts were lower in the severe fibrosis group compared to the mild fibrosis group (p=0.013 and 0.001, respectively).

The number of patients with diabetes or splenomegaly was higher in the severe fibrosis group compared to the mild fibrosis group (p=0.01 and p=0.003, respectively).

TABLE 1: Analysis of the variables.

	Patients with chronic HCV infection (n=201)
Male cases*	93 (46.3%)
Patients with	
Alcohol use*	26 (12.9%)
Steatosis*	67 (49.6%)
Splenomegaly*	20 (10.6%)
HCV genotype 3*	8 (4,4%)
Diabetes*	39 (19.5%)
Patients with IU/mL*	
HCV RNA level >800000	131 (65.2%)
Age, years**	51 (18-77)
Patients who have AST/ALT ratio greater than 1*	41 (20.4%)
Serum HCV RNA level (IU/mLx10^3) **	1940 (2.28-1350000)
Serum ALT level (U/L)**	59 (11-476)
Serum AST level (U/L)**	49 (12-240)
Serum total bilirubin level (mg/dL)**	0.89 (0.2-3.07)
Serum albumin level (g/L)**	4.1 (2.9-5.2)
Serum globulin level (g/ L)**	3.5 (2-5.6)
Serum GGT level (U/L)**	60 (10-588)
Serum ALP level (U/L)**	124 (40-450)
Prothrombin time**	92 (40-158)
Blood thrombocyte count (x1000/mm^3)**	215 (60-592)
Body mass index **	27.18 (16.61-46.09)
Histological activity index**	8 (1-16)
Patients with	
Fibrosis score 0*	18 (9%)
Fibrosis score 1*	101 (50.2%)
Fibrosis score 2*	5 (2.5%)
Fibrosis score 3*	61 (30.3%)
Fibrosis score 4*	16 (8%)

HCV: Hepatitis C virus; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma glutamyl transferase; ALP: Alkaline phosphatase.
* Number of cases (%); ** Mean (range).

TABLE 2: Comparison of patients with mild and severe fibrosis.

	Cases with liver fibrosis stage 0-2 (n = 124; 61.7 %)	Cases with liver fibrosis stage 3-4 (n = 77; 38.3 %)	p
Male patients*	60 (48.4)	33 (42.9)	0.445
Patients with			
Alcohol use*	19 (15.3)	7 (9.1)	0.201
Steatosis*	35 (44.3)	32 (57.1)	0.142
Splenomegaly*	6 (5.3)	14 (18.7)	0.003
HCV genotype 1 and 4*	98 (89.1)	64 (88.9)	0.966
HCV genotype 3*	4 (50)	4 (50)	0,714
Diabetes*	17 (13.8)	22 (28.6)	0.010
Patients with HCV RNA level > 800,000 IU/mL*	81 (65.3)	50 (64.9)	0.955
Age, years **	48 (18-71)	55 (19-77)	0.001
Patients who have AST/ALT ratio greater than 1*	24 (19.4)	17 (22,1)	0.641
Serum HCV RNA level (IU/mlx10^3) **	1510 (6.22-304000)	2760 (2.28-1350000)	0.729
Serum ALT level (U/L)**	49.5 (11-476)	92 (16-458)	0.001
Serum AST level (U/L)**	40 (18-232)	69 (12-240)	0.001
Serum total bilirubin level (mg/dl)**	0.89 (0.2-3)	0.89 (0.27-3.07)	0.334
Serum albumin level (gr/L)**	4.12 (3.2-4.9)	4.1 (2.9-5.2)	0.013
Serum globulin level (gr/ L)**	3.5 (2-4.8)	3.6 (2.6-5.6)	0.017
Serum GGT level (U/L)**	46 (10-254)	67 (18-588)	0.001
Serum ALP level (U/L)**	118 (40-450)	124 (46-437)	0.019
Prothrombin time (%)**	94 (40-158)	88 (61-128)	0.001
Blood thrombocyte count (x 1000/mm^3)**	228.5 (97-417)	193 (60-592)	0.001
Body mass index**	26 (16-46)	27 (19-41)	0.549
Histological activity index**	6.5 (1-12)	11 (6-16)	0.001

HCV: Hepatitis C virus; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma glutamyl transferase; ALP: Alkaline phosphatase.
* Number of cases (%); ** Mean (range).

The logistic regression analysis model revealed that serum GGT and AST levels, age, prothrombin time and splenomegaly were independent variables predictive of advanced liver fibrosis (Table 3). Stage 3-4 liver fibrosis probability of splenomegaly cases compared to stage 0-2 fibrosis was 5 times more (Odds ratio 5.598, p=0.008). Stage 3-4 liver fibrosis probability of the patients compared to stage 0-2 fibrosis was increased 1.861 times with every 10.61 years increase of age, 1.633 times with every 40.37 U/L increase in AST, 1.984 times with every 60.44 U/L increase in GGT (Odds ratio and p value 1.861 and 0.003 for age; 1.633 and 0.018 for AST; 1.984 and 0.013 for GGT, respectively). Stage 3-4 liver fibrosis probability of the patients was decreased 1.848 (1/0.541) times with every 15.27% increase in PTZ (Odds ratio: 0.541, p=0.007).

DISCUSSION

There are numerous publications in the literature investigating factors affecting liver fibrosis in chronic HCV infection.[3-23] These studies suggest longer duration of chronic HCV infection, advanced age, alcohol use, elevated serum ALT or aspartate aminotransferase levels, HCV genotype, ultrasonographic indicators of chronic liver disease, gender and fatty infiltration or elevated histologic activity index in liver biopsy as predictors of the severity of liver fibrosis. In our study, age, elevated serum GGT and AST levels, prolonged prothrombin time and the presence of splenomegaly were found to be independently related to more severe liver fibrosis.

It was reported that the duration of infection was an independent variable increasing liver

TABLE 3: Results of logistic regression analysis for the variables predictive of liver fibrosis.

	Beta value	Odds ratio	95% Confidence Interval (lower and upper limits)	p
Presence of splenomegaly	1.722	5.598	1.583-19.79	0.008
Age, years	0.621	1.861	1.233-2.810	0.003
Serum GGT level	0.685	1.984	1.157-3.405	0.013
Prothrombin time	-0.615	0.541	0.345-0.848	0.007
Serum AST level	0.490	1.633	1.089-2.447	0.018
Constant	-0.482	0.618	-	0.794

GGT: Gamma glutamyl transferase; AST: Aspartate aminotransferase.

fibrosis, however other studies did not support this.[3,5,8,10,11,13,14,19] The date of the patient's first intravenous drug use or transfusion is customarily acknowledged as the start of the HCV infection, which is an unreliable method for determination of the time of infection. However, it is rather difficult in Turkey to determine the duration of chronic HCV infection because of low ratio of drug use as a route of infection in our country.

While some studies suggest that alcohol consumption increases liver fibrosis in chronic HCV infection, it is still controversial.[3-6,8-10,12-14,16,17,20-23] It has been suggested that HCV core protein and alcohol have an additive effect in aggravating lipid peroxidation and synergistically increase the expression of TGF-β and TNF-α in the liver, which results in the activation of hepatic stellar cells and the development of fibrosis.[24] We could not find a significant relationship between alcohol consumption and liver fibrosis. This may be due to retrospective design of our study, and the lack of data regarding the duration and amount of alcohol consumption.[16-23]

There is no consensus for the effect of age in liver fibrosis.[3,5-10,12-14,16-23] While aging seems to extend the duration of chronic HCV infection and thus increases the severity of liver fibrosis, studies demonstrated that the patient's age and the duration of infection independently aggravated fibrosis and indicated that aging affected fibrosis progression through a different pathway.[3,25,26] One study has suggested that antioxidant defense mechanism deteriorates with age, and leaves the liver vulnerable to oxidative stress.[27] Our finding was consistent with the literature.

Studies suggest that histological activity index in liver biopsy has deteriorating or no effect on fibrosis.[4,5,8,10,14,16,17,21,23] In our study, we found higher Knodell scores in patients with more severe fibrosis. The aim of our study was to predict liver fibrosis without a liver biopsy. Because of this, the Knodell score was not included in the multiple regression analysis as a variable predictive of fibrosis.

There are publications supporting and contrasting with the idea that steatosis increases liver fibrosis in chronic HCV infection.[4-6,9,11,12,17-19,21,23] The contentious conclusions might be due to following three reasons: 1) Studies are either cross-sectional or longitudinal and involve repeated liver biopsies: In cross-sectional studies that evaluate a single liver biopsy, the duration of fatty infiltration is unknown. Therefore, longitudinal studies are more powerful. 2) The cause/effect relationship between steatosis and inflammation of the liver is unspecified. 3) Due to factors possibly related to both liver fibrosis and steatosis, such as age, elevated ALT levels, the severity of liver inflammation, high BMI and insulin resistance, it is not known whether liver steatosis has a direct or indirect effect on liver fibrosis. We were unable to ascertain a significant relationship between steatosis and the severity of fibrosis, which we consider to be due to the cross-sectional design of our study and thus the undetermined duration of steatosis. Since this was a retrospective analysis, we did not evaluate the effects of insulin resistance, a mechanism recognized to increase the aggravating effect of liver steatosis on fibrosis. Steatohepatitis and diabetes which may

indirectly be relevant with liver fibrosis were evaluated as variables in our study. However, we could not find a significant relationship between the severity of liver disease and diabetes. In a study on 114 cases, a correlation was found between diabetes and liver fibrosis with univariate analysis, but no correlation was found with multivariate analysis.[27] In this study, when a case group with ALT levels 1.5 times high were evaluated in multi-variate analysis, fibrosis progression was found higher in diabetic cases. However, in this study it is assessed as a major limitation not to include liver steatosis as a variable that can affect liver fibrosis.

Studies showed that BMI values of mild and severe fibrosis groups are not different.[6,8,10,12,13,14,17,19] Although the difference was not significant, we found higher BMI values in patients with severe fibrosis compared to those with mild fibrosis.

Previous studies showed controversial results regarding the relationship between serum HCV RNA levels and liver fibrosis.[8,12,13,16,17,28-30] This discrepancy has been attributed to the intermittent viremia in patients with chronic HCV infection. The lack of relationship between HCV RNA levels and liver fibrosis in our study may be due to a single HCV RNA value. Results on the effect of gender on fibrosis in chronic HCV infection are controversial.[3,4,6-12,16-23] Two studies suggest that male gender has a worsening effect on fibrosis progression.[16,17] Some authors attributed the higher prevalence of severe liver fibrosis in male patients with chronic HCV infection to the inhibitory effect of estrogen on stellate cell proliferation and fibrosis in women.[31] We could not find a significant relationship between gender and liver fibrosis.

Previous studies have demonstrated a lack of correlation between HCV genotype and liver fibrosis.[32] One study determined HCV genotype-3 as an independent predictor of liver fibrosis progression.[17] Since our cases were mostly infected with HCV genotype 1 virus, a healthy evaluation of effect of genotype on liver fibrosis could not be made because genotype 3 infected cases were limited in number.

In one study, it was revealed that the presence of one of the findings as ultrasonographic presence of splenomegaly, hepatomegaly, ascites, esophageal varices or liver heterogeneity were the independent variables affecting the degree of liver fibrosis.[13] In our study, only presence of splenomegaly was included as an ultrasonographic finding and was considered as an independent factor for liver fibrosis.

Serum GGT, total bilirubin, albumin level, prothrombin time and blood thrombocyte count are not independent variables for severe liver fibrosis.[7,8,10-12,14,15,18] Our study showed that elevated serum GGT levels and prolonged prothrombin time were independent variables predicting the severity of liver fibrosis. While chronic liver disease progress to cirrhosis, decreased albumin level and thrombocyte count, and increased bilirubin level are expected findings. In our study, thrombocyte count, serum bilirubin and albumin levels were not detected as variables affecting liver fibrosis, and this may be due to small number of stage 4 fibrosis cases.

Although some studies suggested that elevated ALT, AST levels were common in severe liver fibrosis, some others found no relationship.[3-15,17-19] We found no significant relationship between serum ALT levels and degree of liver fibrosis. Our result may be due to fluctuating ALT levels in chronic HCV infections and only one measurement in our study.

It was demonstrated that in a population of patients with nonalcoholic liver disease, an AST/ALT ratio of 1 or more strongly suggested the presence of cirrhosis.[33] In this study, cirrhosis was defined as stage 4 fibrosis according to Knodell scoring system or clinical existence of portal hypertension or esophageal varices.

A study from our country showed that high AST/ALT ratio was related to liver fibrosis.[34] In this study, chirrosis was defined as stage 5-6 fibrosis according to Ishac scoring system.

Elevation of ALT more than AST in severe liver fibrosis is related to AST presence in the mi-

tochondria, as ALT is in the cytoplasm and more hepatocellular damage is necessary for AST liberation of the cell. Besides, cirrhotic damage of sinusoidal cells with an important role in AST clearing from plasma may contribute to the dominant AST elevation. However, we found that AST/ALT ratio >1 did not affect severe liver fibrosis. In our study, less severe fibrosis degrees such as stage 3 fibrosis were included in the severe fibrosis group. Therefore, we concluded that this might have caused the result that AST/ALT ratio did not affect fibrosis.

Besides, in literature there are some studies claiming that AST/ALT ratio might not be related to cirrhosis on the contrary of accepted opinion, and these are compatible with our study.[35,36]

In conclusion, advanced age, elevated serum GGT and AST levels, prolonged prothrombin time and the presence of splenomegaly are independent variables determining severe liver fibrosis.

Acknowledgement

The authors greatly appreciate Günay Can, MD for statistical analysis.

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