Original Article

Is neutrophil/lymphocyte and platelet/lymphocyte ratio a predictive factor for the fibrous stage in patients with chronic hepatitis B?

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Abstract

Aim: Neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) are an inexpensive inflammatory marker that can be easily obtained from the blood count and can be used in many chronic diseases. In our study, we aimed to investigate the relationship between fibrosis stage and NLR, PLR in patients with chronic hepatitis B virus (HBV).

Materials and Methods: 173 patients with chronic HBV were included in the study. The demographic data and hemogram parameters of all patients were recorded. Histopathological data of the patients were documented. Accompanying comorbid diseases, medications and previous operations were questioned. In chronic HBV patients, fibrosis level was compared with hematological parameters.

Results: A total of 173 patients, 100 (57.8%) of whom were male, were included in the study. As a result of the liver biopsy; mild fibrosis (steage/1-2) in 83(47.9%) patients, moderate fibrosis (steage/3-4) in 35(20.3%) patients and advanced fibrosis (stage/5-6) in 55(31.8%) patients. In advanced fibrosis group, liver function tests and bilirubin levels were significantly higher and albumin level was significantly lower (p<0.05). It was found that NLR level was significantly higher in moderate fibrosis than mild fibrosis, and PLR level was significantly lower in the advanced fibrosis group than in the mild and moderate fibrosis groups (p<0.05).

Conclusions: We showed that there is a significant relationship between fibrosis level and NLR, PLR. However, with the available data, it does not seem possible to say that noninvasive methods can completely replace invasive procedures such as liver biopsy. Prospectively designed studies with larger patient participation are needed.

Keywords: Chronic Hepatitis B; fibrosis; NLR; PLR

INTRODUCTION

Hepatitis B virus (HBV) affects more than 350 million people worldwide and causes chronic hepatitis, hepatocellular cancer, liver cirrhosis, and liver failure (1). Evaluation of the degree of liver fibrosis is very important for diagnosis and treatment in chronic HBVpatients (2). Liver biopsy is the important standard for detecting the level of liver fibrosis, but this invasive method may have classification errors. Non-invasive and cost-effective alternative methods are being investigated due to the patients' hesitation to biopsy (3,4). HBeAg- negative chronic HBV infection constitute the majority of chronic HBV patients. It has been reported that approximately 5% of the world's population is an HBeAg- negative chronic HBV infection (1,4). Non-invasive methods gain importance for closely monitoring the possibility of developing hepatocellular carcinoma and cirrhosis in patients with HBeAg- negative chronic HBV infection (5). Neutrophils and lymphocytes are cells that play a primary role in inflammatory processes, and their number changest emporarily in inflammation. Neutrophil-lymphocyte ratio (NLR) has been reported as a useful index for the diagnosis or prognosis of different disease such as hepatocellular carcinoma, metastatic stomach cancer, acute coronary syndrome, colorectal cancer (6,7). NLR is a non-invasive and inexpensive inflammatory marker that can be easily obtained from a complete blood count. Alkhouri et al. (8) reported that the rate of NLR in individuals with non-alcoholic fatty liver disease is associated with histological changes and can be used to identify patients who are progressive. Kekilli et al. (9) found that the decrease in the rate of NLR in peripheral blood in chronic hepatitis B patients gives high sensitivity, specificity and predictive values in determining the level of

fibrosis. In addition, it has been reported that platelet and platelet lymphocyte ratio (PLR) can be determined easily, quickly and cheaply and are important inflammatory markers for many disease pathophysiology (10-12).

There are limited studies on the role of NLR and PLR in hepatitis B patients (13,14). In our study, we aimed to investigate the relationship between liver damage (fibrosis stage) and NLR and PLR in patients with chronic HBV.

MATERIALS and METHODS

Study Design
The study included 173 patients with the diagnosis of Chronic HBV from the Van Education and Research Hospital gastroenterology and hepatology outpatient clinic between November 2016 and October 2019. Our study was designed retrospectively. Patients who had HBsAg positivity and / or liver function test levels with a high course of six months and who had HBV-DNA positive in the serum sample studied with polymerase chain reaction (Roboscreen, Germany) were evaluated as chronic HBV and liver biopsy was performed. Patients with chronic HBV and volunteers were included in the study. Patients with cigarette use and alcohol use (more than daily alcohol consumption 30 gram for men and 20 gram for women), using anti-inflammatory and antibiotic drugs, coinfection such as Hepatitis C virus (HCV), Hepatitis D virus (HDV), Human immun deficiency virus (HIV), undergoing surgical surgery on the liver, and organ transplantation were not included in the study. In addition, patients with etiology other than HBV that may lead to chronic liver disease, patients with chronic respiratory, renal, cardiovascular, hematological and endocrine problems, pregnant women and individuals with blood transfusions were not included in the study.

Data Assessment
Demographic data (age, gender) of all patients and neutrophil, lymphocyte, platelet, neutrophil / lymphocyte ratio numbers were recorded. Histopathological data of the patients were documented. Accompanying comorbid diseases, medications and previous operations were questioned. In chronic HBV patients, a comparison was made with the level of fibrosis in terms of biochemical and hematological parameters. In addition, according to fibrosis scoring, the sensitivity, specificity, cutt-of and AUC values of biochemical parameters were calculated among patients with mild fibrosis, moderate fibrosis and advanced fibrosis (cirrhosis).

Biochemical and Hematological Measurements
Biochemical parameters were measured from antecubital venous blood samples taken in the morning hours after 8 hours of fasting. Leukocytes, neutrophils, lymphocytes, platelets were measured within 30 minutes from blood samples collected in dipotassium EDTA tubes. In the study, complete blood count analysis was performed with automatic hematology analyzer Beckman Coulter LH 750 (Beckman Coulter; USA). Aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl trans-peptidase (GGT), alkaline phosphatase (ALP), total

protein, albumin, total bilirubin, direct bilirubin, INR and prothrombin time (PT) levels were evaluated.

Liver Biopsy and Histopathological Evaluation
Liver biopsy was performed using ultrasonography guided 16 G biopsy needle. Liver biopsies were sent to the pathology laboratory in 10% formalin. After routine tissue follow-up, tissue samples embedded in paraffin were cut into 5 micron thickness and stained with routine Hematoxylin-eosin (H-E) and Masson trichrome and evaluated under a light microscope. Numerical biopsy material length of less than 1.5 cm and the number of portal areas insufficient for evaluation were excluded. The materials were evaluated by three experienced pathologists without clinical information. Knodell histological activity score was used to perform grade and staging (15). The presence of fibrosis in liver biopsies was made according to the Scheuer score (16). Those with a fibrosis score of 1 and 2 were considered to be low, those with a score of 3 and 4 were moderate, and those with a score of 5 and 6 had advanced fibrosis (cirrhosis).

Ethical Statement
Ethical approval for this study was obtained from the Ethics Committee of our hospital (Date: 07.03.2019 Decision number: 2019/05). All procedures were in accordance with the ethical standards of our institution's human experiment committee and the Helsinki Declaration. Written informed consent forms were obtained from all participants in the study.

Statistical Analysis
The results of our study were analyzed with the program "The Statistical Package for the Social Sciences 19.0 (SPSS Armonk, NY: IBM Corp.)". Data with continuous values were given as mean (± standard deviation), categorical data as frequency and percentage (n,%). The data were tested for compliance with the normal distribution using the kolmogorov-simirnov test, histogram and ± sd. Parametric data of the groups were compared using one-anova test and comparisons between the binary groups were made using the post-hoc test. Chi-square test was used to test categorical data. Cases with $p < 0.05$ were considered statistically significant.

RESULTS

A total of 173 patients, 73 (42.2%) women and 100 (57.8%) men, were included in the study. As a result of the liver biopsy; Mild fibrosis (steage 1-2) in 83 (47.9%) patients, moderate fibrosis (steage 3-4) in 35 (20.3%) patients, and advanced fibrosis (cirrhosis: stage 5-6) in 55 (31.8%) patients. In the demographic comparison between three separate groups made according to the fibrosis stages; While there was no significant difference between sex, the age was found to be significantly higher in advanced fibrosis ($p < 0.05$). In the comparison of liver function tests and bilirubin; it was found to be significantly higher in advanced fibrosis ($p < 0.05$). It was also observed that albumin level decreased significantly in advanced fibrosis ($p < 0.05$). There was no significant relationship between HBV DNA level and fibrosis stage ($p > 0.05$).

In the comparison between some formulas made using hematological parameters and fibrosis levels; NLR level was found to be significantly higher in moderate fibrosis than mild fibrosis, and PLR level was significantly lower in the advanced fibrosis group than in the mild fibrosis and moderate fibrosis groups (p <0.05) (Table 1).

In our ROC analysis to distinguish mild fibrosis from moderate fibrosis, AUC for NLR was 0.637, and when taken as 1.55 cut-off, specificity and sensitivity were 71.4% to 45.8%, respectively. In our ROC analysis to distinguish moderate fibrosis from cirrhosis, AUC for PLR was found to be 0.842, and when taken as 76.96 cut-off, the specificity and sensitivity were 82.6% to 81.5%, respectively. In our ROC analysis to distinguish moderate fibrosis from cirrhosis, AUC was found to be 0.904 for platelets, and when 146.5 was taken as cut-off, specificity and sensitivity were 97.1% to 79.6%, respectively. In our ROC analysis to distinguish the moderate fibrosis from cirrhosis, AUC for leukocyte was found to be 0.812, and when 5.97 was taken as cut-off, the specificity and sensitivity were found to be 82.9% - 68.5%, respectively.

Table 1. Comparison of fibrous stage with demographic, laboratory and histopathological data in chronic HBVpatients				
	Mild Fibrosis (n=83)	Moderate Fib-rosis (n=35)	Advenced Fib-rosis (n=55)	P value
Gender (M/F)	41/42	21/14	38/17	0.069
Age	41.9±13.6[a]	50.9±11.7[b]	56.3±10.3[b]	**<0.001**
AST	27.5±16.9[a]	43.3±46.7[b]	47.3±40.2[b]	**0.001**
ALT	36.3±32.3	53.4±65.8	36.5±29.9	0.088
ALP	83.55±27.4[a]	83.27±33.55[a]	111.4±53.4[b]	**<0.001**
GGT	18.4±10.56[a]	33.44±32.26[a]	77.69±90.87[b]	**<0.001**
Albumin	4.40±0.44[a]	4.23±0.40[a]	3.37±0.68[b]	**<0.001**
T. bilirubin	0.57±0.28[a]	0.84±0.44[a]	2.45±3.50[b]	**<0.001**
D. bilirubin	0.20±0.09[a]	0.28±0.13[a]	2.45±3.4[b]	**<0.001**
HBV DNA	180.25±931.5	50.00±172.15	3.95±17.16	**0.293**
Leukocyte (x10³)	6.99±1.54[a]	7.32±1.57[a]	5.04±2.32[b]	**<0.001**
Neutrophil (x10³)	3.94±1.13[a,b]	4.36±1.55[a]	3.6±1.2[b]	**0.023**
Lymphocyte(x10³)	2.43±1.13	2.14±0.48	2.24±1.01	0.119
Platelet (x10³)	239.3±59.68[a]	225.7±69.4[a]	103.3±59.0[b]	**<0.001**
NLR	1.68±0.49[a]	2.13±0.89[b]	1.77±0.66[a]	**0.003**
PLR	102.92±30.98[a]	111.0±43.79[a]	61.34±66.84[b]	**<0.001**

DISCUSSION

Hepatitis B infection is a common problem all over the world and in our country and may lead to serious complications in the future (14,17). Aygun et al. reported that the age was higher in the group with high fibrosis statistically significantly in patients with hepatitis B (17). Atay et al. also found that the mean age in the group with advanced fibrosis level was higher in their study (14). In our study, in our comparison between the demographic data and the degree of fibrosis, we found that while there was no significant difference between gender and the degree of fibrosis in parallel with the available data, the age was significantly higher in the moderate and advanced fibrosis.

In patients with chronic HBV, although it is a gold standard liver biopsy in determining the degree of liver fibrosis, it causes some complications as it is an invasive procedure. For this reason, many non-invasive methods are tried to be developed as an alternative to liver biopsy (14). Imaging methods such as elastography (fibroscan) and some formulations such as aspartate transaminase to platelet ratio index (APRI), fibrosis index based on 4 factors (FIB-4), the aspartate aminotransferase (AST) / alanine aminotransferase (ALT) ratio (AAR), and the AAR / platelet ratio index (AARPRI) are used. Transient elastography (TE; FibroScan, Echosens, France) is a novel technology to diagnose liver fibrosis, which is based on the assessment of liver stiffness measurement (LSM) using ultrasound and low-frequency elastic waves. FibroScan has high sensitivity, specificity, and accuracy in detecting cirrhosis. Many studies have assessed the diagnostic performance and accuracy of fibroscan in detecting cirrhosis, with specificity and sensitivity being reported to approach 90% (18,19). Similarly, NLR and PLR are used to determine the stage of liver fibrosis. White spheres play a role in the pathogenesis of various diseases. In addition to increases in neutrophil count in acute inflammatory processes, a decrease in lymphocyte count due to acute stress reflects changes in the immune system. The ratio of the set who subgroups to each other is used as an inflammation marker (9,10). Similarly, the number of platelets is affected by inflammatory processes and the level of fibrosis in the liver. NLR and PLR are easily accessible and inexpensive inflammatory markers that can be obtained from a complete blood count. Many studies have evaluated NLR

and PLR as a noninvasive diagnostic model of hepatic fibrosis in different chronic liver diseases. Recent studies have reported that NLR is practical for predicting fibrosis and prognosis in patients with chronic hepatitis B, C and NASH (9,20,21). Yılmaz et al. found that NLR levels were significantly lower in patients with fibrosis 2 and above in adult HBeAg- negative chronic HBV infection (5). Kekilli et al. found that the decrease in peripheral blood NLR ratio gives high sensitivity, specificity and predictive values in hepatitis B patients with advanced fibrosis, and suggested that NLR is a new marker of non invasive fibrosis in patients with CHB (9). In another group of studies, the researchers reported that the cut-off value of 2.36 predicted a lower mortality rate among patients with hepatitis B, and a value higher than 6.12 predicted a high risk of death (22). However, Celikbilek et al. there was no significant difference in NLR value between adult chronic hepatitis B patients and the control group (23). Similarly, Uluca et al. could not detect a significant relationship between NLR and fibrosis (24). Although there are studies showing that low platelet values are associated with advanced hepatic fibrosis, some studies have shown opposite findings (4,25). Therefore, it is controversial whether NLR and PLR values fully reflect hepatic fibrosis in these patients. In our study, it was seen that NLR level was significantly higher in moderate fibrosis compared to mild fibrosis and PLR level was significantly lower in the advanced fibrosis group compared to mild fibrosis and moderate fibrosis groups.

LIMITATIONS

Our study has some strengths and limitations. The study was designed retrospectively. For this reason, some conditions such as medication and infection that may affect the level of hematological parameters may be unregistered and may cause an error in somedata. However, the strengths of our study are the presence of histopathological data in all our patients and the inclusion of a relatively large sample.

CONCLUSION

In conclusion, we showed that there is a significant relationship between fibrosis level and NLR and PLR in our study. However, with the available data, it does not seem possible to say that non invasive methods can completely replace invasive procedures such as liver biopsy. In the light of these findings, there is a need for prospectively designed studies with wider patient participation to help theese parameters, together with other non invasive parameters, to identify individuals at high risk of advanced and progressive disease.

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