Araştırma Makalesi / Research Article

Can neutrophil / lymphocyte ratio be used as a marker of fibrosis in chronic Hepatitis C?

Nötrofil/lenfosit oranı kronik Hepatit C hastalarında fibrozis belirteci olarak kullanılabilir mi?

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Abstract

Background: It is reported that about 170-200 million people are chronically infected with Hepatitis C virus (HCV) in the world. In developed countries, about 25% of cirrhosis cases, one third of hepatocellular cancers, and 40% of all chronic hepatitis cases are caused by HCV. In this study, we want to determine the utility of neutrophil/lymphocyte ratio (NLR) to predict the fibrosis in patients with chronic hepatitis C.
Methods: In our study NLR was prospectively investigated whether it can predict, or not the fibrosis on patients who diagnosed Chronic hepatitis C (CHC) and underwent liver biopsy in July 2011 - January 2013 by Kayseri Training and Research Hospital, Department of Gastroenterology.
Results: 81 patients diagnosed with CHC and underwent liver biopsy were included in the study. Fibrosis and necroinflammatory activity in liver biopsy specimens was determined in accordance to Ishak fibrosis scoring system. All Ishak scores were re-scored according to France METAVIR Cooperative Study Group as Score 0, 1, 2, 3, 4. In such re-scored METAVIR scores, Score 0, 1, 2 were defined as low fibrosis and Score 3, 4 were defined as high fibrosis. 46 of 81 patients (% 57) were in low fibrosis group and 35 of them (43%) were in high fibrosis group. When Comparing the NLRs; it is seen that in low fibrosis group NLR was 2.09 and in high fibrosis group NLR was 1.72 (p=0.038). It is seen that AST predicts fibrosis because of AST/ALT ratio was higher due to increase in favor of AST especially in patients with cirrhosis. Furthermore, NLR predicts fibrosis with 60% sensitivity and 65.2 % specificity if cutt off value is taken as 1.77.
Conclusion: According to our results, NLR can predicts the degree of liver fibrosis in chronic liver disease, despite it's lower sensitivity and specificity.

Keywords: Neutrophil/Lymphocyte Ratio, Fibrosis, Hepatitis C

Öz.

Amaç: Dünya nüfusunun yaklaşık olarak %3'ü Hepatit C virusu (HCV) ile enfekte olup, 170-200 milyon insanın da HCV ile kronik enfekte olduğu rapor edilmektedir. Gelişmiş ülkelerde, siroz vakalarının %25 kadarı, hepatosellüler kanserlerin üçte biri, tüm kronik hepatit vakalarının % 40'ının sebebi HCV'dir.
Bu çalışmada da nötrofil/lenfosit oranının (NLO) Kronik Hepatit C'li (KHC) olgularda fibrozisin düzeyini gösterebilecek bir marker olarak kullanılıp kullanılamayacağını araştırmak istenmiştir.
Materyal ve Metod: Temmuz 2012- Ocak 2013 tarihleri arasında Kayseri Eğitim Araştırma Hastanesi Gastroenteroloji Kliniği'nce KHC tanısı konularak karaciğer biyopsisi uygulanan hastaların bakılan tam kan sayımındaki NLO'nun kontrol grubunun NLO'su ile karşılaştırılması suretiyle karaciğer biyopsisinde fibrozis skorunun ciddiyetini ön görüp göremeyeceği prospektif olarak araştırılmıştır.
Bulgular: KHC tanısıyla biyopsi uygulanan 81 hasta çalışmaya alındı. Karaciğer biyopsi örneklerinde fibroz ve nekroinflamatuar aktivite, İshak fibroz skorlama sistemine göre belirlendi. Tüm Ishak skorları Fransa METAVIR Kooperatif Çalışma Grubu'na göre Puan 0, 1, 2, 3, 4 olarak tekrar puanlandı. Bu tekrar puanlanan METAVIR puanlarında Puan 0, 1, 2 düşük fibroz ve Puan 3, 4 ise yüksek fibrozis olarak tanımlandı. 81 hastanın 46'sı (% 57) düşük fibrozis ve 35'i (% 43) yüksek fibrozis grubundaydı. NLO'ları Karşılaştırırken; Düşük fibrozis grubunda NLO'nun 2.09, yüksek fibrozis grubunda NLO'nun 1.72 (p = 0.038) olduğu görüldü. AST'nin, özellikle sirozlu hastalarda AST / ALT oranının AST lehine artışı nedeniyle fibrozu öngördüğü görülmüştür. Ayrıca, NLO, kesim değeri 1,77 olarak alındığında % 60 duyarlılık ve % 65,2 özgüllük ile fibrozisi öngördüğü görülmektedir.
Sonuç: Çalışmamızın sonuçlarına göre, Nötrofil Lenfosit Oranı, kronik karaciğer hastalığı olan hastalarda karaciğer fibrozunun derecesini tahmin edebilir.

Anahtar Kelimeler: Nötrofil / Lenfosit Oranı, Fibrozis, Hepatit C

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Introduction

Hepatitis C virus (HCV) is a member of Flaviviridea family and carries ribonucleic acid as its genetic material. HCV is an enveloped, non-cytopathic, hepatotrophic virus which may lead to acute and chronic necroinflammatory hepatitis and can cause serious health problems. In developed countries, about 25% of cirrhosis cases, one third of hepatocellular cancers, and 40% of all chronic hepatitis cases are caused by HCV (1). Virus has been identified for the first time in the late 1970s as a result of failure to demonstrate Hepatitis A virus (HAV) and Hepatitis B Virus (HBV) in cases of transfusion associated hepatitis and was named as non-A, non-B hepatitis virus (2).

The typical histological features of chronic hepatitis C (CHC) consist of varying degrees of hepatocellular necrosis, inflammation and fibrosis. While inflammation is an indicator of activity, fibrosis is an indicator of staging. Disease activity may fluctuate, worsen over time and exhibit improvements, but it is believed that fibrosis is progressive and the major cause of liver remodeling and progression of chronic liver disease and cirrhosis. For these reasons, the degree of fibrosis determines the natural course of CHC (3).

The leukocyte count and the ratio of its subtypes are considered as a marker of inflammation. Neutrophil/lymphocyte ratio (NLR), has been shown to be an indicator of clinical course in heart failure, stable angina pectoris, acute coronary syndrome (4), colorectal cancer (5), lung cancer (6), non-alcoholic fatty liver disease (NAFLD) (7) and in cases where inflammation plays an important role in its etiology. In this study, we aimed to investigate if NLR can be used as a marker of fibrosis in CHC patients.

Material and Methods

Between July 2011 - January 2013, 81 newly diagnosed CHC patients were included in the study who diagnosed by Kayseri Training and Research Hospital, Department of Gastroenterology. Liver biopsies were performed on these CHC patients. Biopsy specimens were examined by Kayseri Training and Research Hospital Pathology Laboratory by a single pathologists. The patients who was agreed to participate in the study, previously untreated and was over the age of 18 were included in the study. Patients who did not agree to participate in the study, previously treated with interferon and/or antiviral therapy, acute hepatitis C patients, had diseases may affect the NLR such as severe concurrent diseases, infectious diseases, hypothyroidism, hyperthyroidism and heart failure, who had NAFLD or infected with HBV and had disease goes with hepatocyte injury such as Wilson's disease, hemochromatosis and alpha-1 antitrypsin deficiency were excluded from the study.

Patients' ages, genders, HCV RNA levels, neutrophil-lymphocyte ratios, alanine aminotransferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) levels from serum which obtained the day of biopsy were recorded to the statistical analysis program. On the day of blood taken, percutaneous liver biopsy was performed to the patients, by using a 16-Gauge liver biopsy needle.

Liver fibrosis and necroinflammatory activity in biopsy specimens was determined according to Ishak liver fibrosis scoring system. These Ishak scores were converted to METAVIR scoring system which is determined according to the France METAVIR Cooperative Study Group's Chronic Liver Disease Histological Scoring System. In this scoring system, score 0 meets no fibrosis; Score 1 is star-shaped expansion in portal region without septal formation; Score 2 means some expansion areas with rare septal formations; Score 3 is the presence of a large number of septal formation and also score 4 meets cirrhotic liver (8). In such re-scored METAVIR scores, Score 0, 1, 2 were defined as low fibrosis group and Score 3, 4 were defined as high fibrosis group. These re-grouped fibrosis scores were recorded to the statistical analysis program which contains demographic and biochemical data of patients.

Statistical Analysis: For statistical analysis, the statistical package for the social sciences (SPSS version 25 Inc., Chicago, Illinois) was used. Conformity to normal distribution of data were evaluated with *Shapiro-Wilk test*, histogram and q-q graph plotting. Logarithmic transformation was performed to the HCV-RNA variable due to its discrete and extremely skewed form. Independent samples T-test and Mann-Whitney U tests were evaluated for quantitative variables and chi-square test were evaluated for qualitative variables. Results were expressed as percentage for frequency, standard deviation for mean and quarters for medians. Also, single and multiple logistic regression analysis were performed and odds ratios with 95% confidence intervals were calculated. According to results of single analysis in $p < 0.10$ level, variables were included in multiple models and backward elimination method was applied to determine independent risk factors. In addition, ROC analysis was performed to determine the performance in predicting fibrosis for NLR, AST, ALT and GGT variables. The areas under the ROC curves along with 95% confidence intervals were calculated and they were compared with each other. The threshold values were determined for each factor and for these values sensitivity, specificity, positive and negative predictive values were calculated and *kappa tests* was performed. Data analysis was performed in R 3.0.0 program. $p < 0.05$ was considered as statistically significant.

Results

Of these 81 CHC patients, 23 were male (28.4%) and 58 were women (71.6%). Among these patients with CHC mean age was 55.31 ± 3.10, average AST value was 46.00 (29.00-67.00) IU / L, average ALT value was 50.00 (33.00-81.00) IU / L, average ALP was 89.00 (76.00-112.00) IU / L and average GGT value was 50.00 (32.00-108.00) IU / L, and average of NLR was 2.00 (1.47-2.60).

Also among CHC patients, two groups were created; low fibrosis and high fibrosis group. Age, sex, NLR, AST, ALT, ALP, GGT, hepatitis activity index (HAI) and logarithmic HCV-RNA level comparisons are shown in Table 1. Age and gender of CHC patients were similar in both groups (p=0.043 vs 0.335). Average value of NLR was 2.09 (1.67-2.87) in low fibrosis group and 1.72 (1:18 to 2:54) in high fibrosis group [p = 0.038; Odds Ratio: 0.66 (12:41 to 1:05)]. According to AST, ALT, ALP and GGT values of low fibrosis and high fibrosis groups, AST and GGT values were higher in high fibrosis group [AST; p = 0.004; Odds Ratio: 1.01 (1:00 to 1:01) and GGT; p=0.001; Odds Ratio: 1.01 (1:00 to 1:01)].

Mean HAI was 7.00 (5.00 to 8.00) in low fibrosis group and 8.00 (7.00 to 8.00) in high fibrosis group [p < 0.001; Single Odds Ratio: 1.80 (1.25-2.60) and Multiple Odds Ratio: 1.80 (1.25-2.60)]; In comparing to logarithmic HCV-RNA levels it was 5:40 ± 0.80 copies/ml in low fibrosis group and 5.78 ± 1.12 copies/ml in high fibrosis group [p=0.077; Odds Ratio: 1.63 (0.93-2.84)].

The fibrosis predicting forces of AST, ALT, NLR and GGT variables in patients with CHC were compared in Figure 1a, and the area under the ROC curves were obtained as 0.67 (0.56-0.77) for AST, 0.59 (0.48-0.70) for ALT, 0.64 (0.52-0.74) for NLR and 0.72 (0.60-0.81) for GGT. In comparison of area under the curves, the areas between the AST/ALT and ALT/GGT were statistically significant (p <0.05), other binary comparisons were not significant (p> 0.05). In figure 1b, NLR variable's distribution for the 1.77 cut-off value is seen in low and high fibrosis groups. In this figure, it is seen that NLR predicts fibrosis with a 60.0% sensitivity and 65.2% specificity if it is ≤ 1.77 (K=0.250 ve p=0.024).

When viewed the performance measurements of the diagnostic tests for determining the fibrosis in CHC patients; if AST≥ 45 IU/L the sensitivity 0.71 (0.54-0.85), specificity 0.63 (0.48-0.77), positive predictive value of 0.60 (0.43-0.74), and negative predictive value of 0.75 (0.58-0.87) was observed (K = 0.337 and p = 0002); if GGT ≥ 45 IU/L the sensitivity 0.79 (0.61-0.91), specificity 0.64 (0.49-0.78), positive predictive value was 0.62 (0.45-0.77), and negative predictive value of 0.81 (0.64-0.92) was observed (K=0.417 and p<0001) (Table 2).

Discussion

The degree of liver fibrosis is important for determining the clinical course of chronic liver disease, selection of patients for specific treatments and success of treatment. Liver biopsy is the gold standard invasive method for histological diagnosis and staging of fibrosis which has high costs and rare but serious complications (9). Therefore, in recent times interest for the detection of liver fibrosis based on serum non-invasive methods are raised. In this way, many researchers have developed some diagnostic tests. Despite the growing non-invasive tests and imaging techniques, we do not have a non-invasive test that can be used reliably for staging liver fibrosis in CHC and the other viral hepatitis (10).

NLR was first studied in septic critical care patients as a predictive marker in terms of disease severity and clinical course (11). Patients who have increased NLR were found to be worse disease in colorectal cancer (5). Patients have higher preoperative NLR, were found to have poorer survival in coronary artery bypass surgery (12). Alkhouri and colleagues found that increased NLR predicts advanced fibrosis in NAFLD (13). Also, Karaman et al revealed that polyps in patients with increased NLR is tend to be more neoplastic (14).

In contrast to the novel findings of Coskun BD et al (15) and Meng X et al (16), it was thought that NLR can be used to predict liver fibrosis especially after HCV infection according to findings of Abdel-Razik A et al (17). In this paper it is shown that NLR is a reliable marker to predict insulin resistance and fibrosis stage in CHC virus infection and patients with advanced fibrosis had an elevated NLR compared with patients with low fibrosis stages.

In our study AST was significantly higher in high fibrosis group than low fibrosis group as consistent with the literature. Likewise, GGT and log (HCV-RNA) levels were also statistically significantly higher in high fibrosis group than low fibrosis group. It is observed that GGT elevation indeed is used as a marker for prediction of fibrosis and through the power of prediction of advanced fibrosis, it has found its place in various combined tests like Fibrotest® (18). Limited information is available in the literature about HCV viral load for its impact to hepatic fibrosis, but it is seen that high viral load does not effect the hepatic fibrosis in a few studies (19). But in our study, patients have high viral load seems to have higher fibrosis scores.

In our study, the average NLR value was 2.09 (1.67-2.87) in low fibrosis group of and 1.72 (1:18 to 2:54) in high fibrosis group, and in the case NLR ≤ 1.77 it seems to be predicted advanced fibrosis with 60.0% sensitivity and 65.2% specificity (Table 1 and Figure 1b). Also, as seen in Table 2, while NLR ≤ 1.77, the positive predictive value of 0.57 (0.40-0.73) and negative predictive value of 0.68 (0.52-0.81) was observed in predicting advanced fibrosis (95% CI).

Table 1. CHC patients' fibrosis scores and comparison of the results of the logistic regression analysis

Variable	Group Comparisons			Logistic Regression Analysis	
	Low Fibrosis (*n*=46)	High Fibrosis (*n*=35)	*p*	Single OR (95% CI)	Multiple OR (95% CI)
Age (years)	53.35±10.40	57.89±9.02	0.043	1.05 (1.00-1.10)	-
Gender (Male/Female)	15 (32.6)/31 (67.4)	8 (22.9)/27 (77.1)	0.335	1.63 (0.60-4.45)	-
NLR	2.09 (1.67-2.87)	1.72 (1.18-2.54)	0.038	0.66 (0.41-1.05)	-
AST (IU/L)	35.50 (26.00-55.00)	53.00 (38.00-89.00)	0.004	1.01 (1.00-1.01)	-
ALT (IU/L)	43.50 (31.00-81.00)	52.00 (43.00-96.00)	0.157	1.01 (1.00-1.01)	-
ALP (IU/L)	84.00 (76.00-101.00)	96.00 (84.00-117.00)	0.130	1.01 (1.00-1.01)	-
GGT (IU/L)	36.00 (27.00-56.00)	75.00 (49.00-122.00)	0.001	1.01 (1.00-1.01)	-
HAI	7.00 (5.00-8.00)	8.00 (7.00-8.00)	<0.001	1.80 (1.25-2.60)	1.80 (1.25-2.60)
Log (HCV-RNA) copies/mL	5.40±0.80	5.78±1.12	0.077	1.63 (0.93-2.84)	-

Table 2. Performance measurements of the diagnostic tests for determining the fibrosis in CHC patients and kappa test results

Variable	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	*K*	*p*
NLR **(≤1.77)**	0.60 (0.42-0.76)	0.65 (0.50-0.79)	0.57 (0.40-0.73)	0.68 (0.52-0.81)	0.250	0.024
AST **(≥ 45 IU/L)**	0.71 (0.54-0.85)	0.63 (0.48-0.77)	0.60 (0.43-0.74)	0.75 (0.58-0.87)	0.337	0.002
ALT **(≥ 42 IU/L)**	0.77 (0.60-0.90)	0.50 (0.35-0.65)	0.54 (0.39-0.68)	0.74 (0.55-0.88)	0.258	0.013
GGT **(≥45 IU/L)**	0.79 (0.61-0.91)	0.64 (0.49-0.78)	0.62 (0.45-0.77)	0.81 (0.64-0.92)	0.417	<0.001

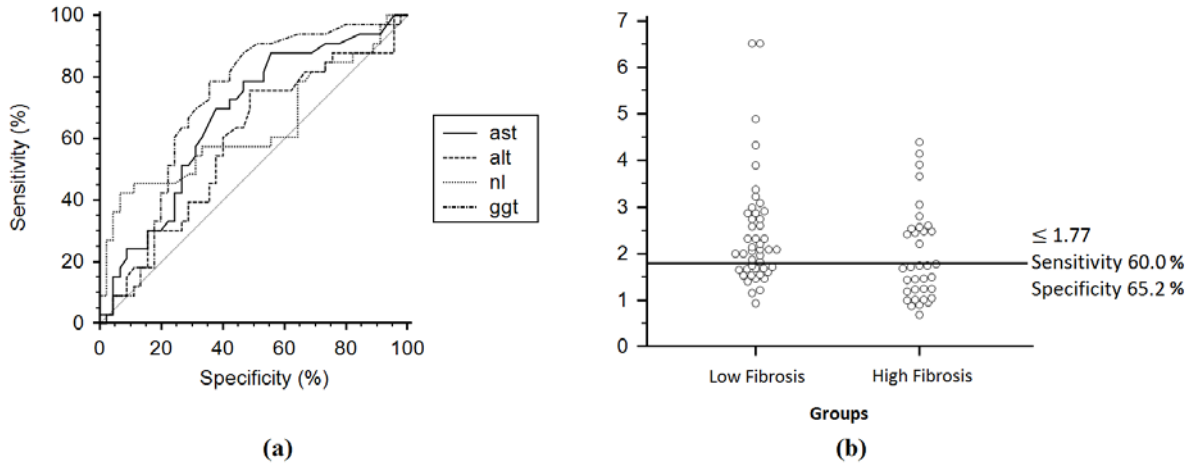

Figure 1. (a) Comparison of ROC curves of AST, ALT, GGT and NLR variables in predicting fibrosis in CHC patients (b) NLR variable's distribution for low and high fibrosis in cutoff value of 1.77

In many diseases NLR's predictive power is valid when it is high. But in our study it is observed that NLR is available for prediction of advanced fibrosis when it is lower than 1.77 threshold. This condition might be caused from changing of leukocyte formula in advanced fibrosis in CHC. Nonetheless, despite the detailed literature review, no article was found about how leukocyte formula is affected in patients who have advanced fibrosis and/or cirrhotic liver. Although there are contradictory studies in the literature, as in our study, we think that new studies are needed about cirrhosis and advanced fibrosis associated changes in the leukocyte formula and NLR. Also there is a need to identify that if NLR can be used for predicting fibrosis stage in CHC virus infections.

Conclusion

In our study, it is observed that, despite its low sensitivity and specificity, NLR can predict advanced liver fibrosis in CHC patients. NLR is an easy and cheaper method than many other predictive methods used in CHC patients. NLR may be useful for combination tests in coming years.

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