The Neutrophil-to-Lymphocyte Ratio as A Noninvasive Marker in Patients with Biopsy-Proven Non-Alcoholic Steatohepatitis

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Objective: Non-alcoholic fatty liver disease is a global health problem with an increasing prevalence. The neutrophil-to-lymphocyte ratio is a cheap inflammatory parameter that can be easily calculated from routine complete blood count tests. This study was designed to investigate the neutrophil-to-lymphocyte ratio in patients with non-alcoholic steatohepatitis and simple hepatosteatosis.

Methods: Fifteen patients with biopsy-proven non-alcoholic steatohepatitis, 65 patients with simple steatosis diagnosed with abdominal ultrasound, and 65 healthy controls were included. Anthropometric measurements were obtained during a routine physical examination. The neutrophil-to-lymphocyte ratio was calculated from routine complete blood count tests, and its relationship with various clinical and laboratory parameters was analyzed.

Results: The mean neutrophil-to-lymphocyte ratio was 2.16±0.49 in the patients with non-alcoholic steatohepatitis, 1.62±0.43 in the patients with simple steatosis, and 1.51±0.31 for healthy controls; the difference among the groups of patients was statistically significant (p<0.001). A paired analysis revealed that patients with non-alcoholic steatohepatitis had a significantly higher neutrophil-to-lymphocyte ratio than patients with simple steatosis and healthy controls, whereas the difference between the latter two groups of patients was not statistically significant. The neutrophil-to-lymphocyte ratio was not associated with the degree of steatosis on performing abdominal ultrasound and with histological findings of liver biopsies (p>0.05). ROC analyses for the neutrophil-to-lymphocyte ratio to differentiate patients with steatohepatitis revealed an AUC of 0.868 (95% CI: 0.781–0.956) and 86.5% sensitivity and 81% specificity for the selected cut-off value of 1.793.

Conclusion: The results of this study showed that the neutrophil-to-lymphocyte ratio was higher in patients with steatohepatitis than in patients with simple steatosis and healthy controls. Taking into account that the difference between patients with simple steatosis and healthy controls was not statistically significant, the increased neutrophil-to-lymphocyte ratio in the patients with steatohepatitis can be attributed to a low level of systemic inflammation accompanying the hepatic inflammation.

Keywords: Neutrophil-to-lymphocyte ratio, non-alcoholic fatty liver disease, steatohepatitis

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a major health problem worldwide. While varying among countries, the prevalence of NAFLD is steadily increasing globally and particularly in developed countries (1). Its prevalence is estimated to be around 20–30% in Western countries and 5–18% in Asia, and its worldwide prevalence is estimated to be 24.4% (2, 3). The NAFLD spectrum ranges from a benign clinical picture, such as simple liver steatosis, to inflammation, hepatocyte damage, and non-alcoholic steatohepatitis (NASH) that is characterized by fibrosis, cirrhosis, and hepatocellular carcinoma development. Currently, NAFLD is one of the most common causes of liver cirrhosis and related liver transplants in developed countries (4). Although the pathogenesis of NAFLD is not well known, there are reports suggesting that intestinal metabolic products, microbiota, various immunological mechanisms, pro-inflammatory mediators released from the adipose tissue, and various cytokines play a role in the pathogenesis (5, 6). Although the pathogenesis is not fully known, risk factors associated with NAFLD have been well defined. In particular, the association of metabolic syndrome and its components (obesity, hypertension, dyslipidemia, and type 2 diabetes mellitus [DM]) with NAFLD has been shown in the literature (7). In the NAFLD spectrum, NASH patients have a discriminatory significance for the prognosis because unlike simple liver steatosis, NASH is characterized by progressive inflammation and fibrosis and has the potential to progress to cirrhosis. However, unfortunately, there are currently no optimal noninvasive diagnostic methods with high specificity and sensitivity that can be used to diagnose NASH patients. Imaging methods demonstrate liver steatosis, but these methods are unable to demonstrate inflammation and early-stage fibrosis. There are no validated biochemical markers that can distinguish NASH patients from simple steatosis patients. For these reasons, liver biopsy in diagnosing NASH is still considered as the gold standard. Although liver biopsy is a low-risk procedure, it can cause many complications, particularly hemorrhage and even death (albeit rarely), due to the fact that liver biopsy is an invasive procedure.
The neutrophil-lymphocyte ratio (NLR) is an inexpensive inflammatory indicator that can be calculated with a simple blood count. There are publications in the literature showing that the NLR is associated with various inflammatory diseases, many cancers, and various liver diseases (8-10). In this study, we aimed to investigate the relationship of NLRs in NAFLD with various clinical and laboratory parameters.

**Methods**

A total of 145 subjects, including 15 NASH patients diagnosed by liver biopsy (group 1), 65 simple liver steatosis patients diagnosed by abdominal ultrasonography (group 2), and 65 healthy controls (group 3) were included. The study was approved by the Necmettin Erbakan University Meram School of Medicine Ethics Committee. Written informed consent was obtained from the included subjects, and volunteer consent forms were signed. The NASH group consisted of patients with an elevated hepatic function test lasting longer than 6 months, with no other liver or biliary tract disease, with hypechogenic liver on ultrasonography, and whose NASH diagnosis was confirmed by liver biopsy. Patients with normal serum transaminase levels and patients in whom hypechogenic and fatty livers were detected by abdominal ultrasonography were included in the simple liver steatosis group. According to the findings of abdominal ultrasonography, the grading of liver steatosis has been defined as grade 0=no steatosis; grade 1=slight diffuse echogenicity increase and normal diaphragm and intrahepatic vessel echogenicity; grade 2=moderate echogenicity increase, slight graying in intrahepatic vessel echogenicity, and diaphragmatic echogenicity; and grade 3=marked increase in liver echogenicity with imperceptible periporal and hepatic venous echogenicity and obscuration of diaphragm. Healthy individuals who had normal serum transaminase levels, no liver fattening on abdominal ultrasonography, and had no known systemic disease were included in the control group. Other possible chronic liver diseases were eliminated in patients in the NASH and simple steatosis groups by examining the hepatitis B surface antigen, hepatitis B core antigen, anti Hepatitis C virus antibody, antinuclear antibody, anti-smooth muscle antibody, anti-liver-kidney microsomal antibody, serum copper and ceruloplasmin levels, and transferrin saturation. Patients with known liver disease, who consumed alcohol (>20 g/day for men, >10 g/day for women), with known cancer, and with autoimmune disease or an active infection were excluded. Anthropometric measurements were taken while a routine physical examination was performed in the patient and control groups. Patients' body weights were measured in light clothing using calibrated scales. The body mass index (BMI) was calculated using the formula “body weight (kg)/height (m)2.”

Blood samples were taken from the subjects on an empty stomach in the morning. Fasting blood glucose, bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyltransferase (GGT) levels were measured using an autoanalyzer (Abbott Architect 16000, Abbott Laboratories, Abbott Park, IL, USA) and the hemograms were studied using automatic Coulter devices.

The AST-to-platelet ratio index (APRI) and fibrosis-4 (FIB-4) scores, as noninvasive fibrosis scores, for all three groups were calculated using the following formulae: APRI=(AST/AST upper limit of normal)/(Plt (10^9/L)×100 and FIB-4=[age (years)×AST (U/L)]/[Plt (10^9/L)×(ALT (U/L))^2]."

**Statistical analysis**

The Statistical Package for the Social Sciences 19.0 (SPSS Armonk, NY: IBM Corp.) package program was used for statistical analysis. Continuous variables were expressed as mean±standard deviation and categorical data as frequency and percentage (%). In order to test for the significance of differences between three or more groups, one-way ANOVA test was used when tested parameter was normally distributed, and Kruskal Wallis test was used when the distribution was not normal. When the difference was statistically significant, the two groups were compared using the independent sample t-test for variables with normal distribution and the Mann-Whitney U test for variables without normal distribution. ROC curves were used to determine cut-off values associated with the tested variables and to calculate the sensitivity and specificity. Negative and positive predictive values were calculated for the determined cut-off values. p<0.05 was considered statistically significant.

**Results**

The mean age of the study group was 49.0±13.0 years. There was no statistically significant difference between the groups in terms of age and sex distribution. Fasting blood glucose, AST, ALT, and GGT levels were significantly higher in the NASH group when compared to the simple steatosis group and healthy control group. While 76.4% of the patients had DM and 52.9% of them had hypertension in the NASH group, there was DM in 7.7%, impaired fasting glucose in 27.7%, and hypertension in 13.8% of the simple steatosis group. Clinical, laboratory, and demographic characteristics of the study groups are summarized in Table 1.

When analyzed in terms of the anthropometric measurements, the BMI and waist-hip ratios of the patients in the NASH and simple steatosis groups were significantly higher than the healthy controls (p=0.005 and p<0.001, respectively). When the NASH and simple steatosis groups were compared, while there was no difference between the two groups in terms of BMI (p=0.22), the waist-hip ratio was observed significantly higher in the NASH group (p=0.04).

The mean NLR was 2.16±0.49 in the NASH group, 1.62±0.43 in the simple steatosis group, and 1.51±0.31 in the healthy controls, and the difference among the three groups was statistically significant (p<0.001). It was found in paired comparisons that the NLR was significantly higher in NASH patients than both the simple steatosis and healthy control groups (p<0.001 for both), but there was no difference between the simple steatosis and healthy control groups (p=0.086). The NLR in all three groups is given in Figure 1.

The liver biopsy findings of patients in the NASH group are summarized in Table 2. There was no significant relationship between the NLR and histological findings in the liver biopsy (ballooning, steatosis grade, inflammation and fibrosis grade, etc…) (p>0.05).

When analyzed in terms of abdominal USG findings, while steatosis grade 1 was found in 8 patients (53.3%), steatosis grade 2 in 3 patients (20%), and steatosis grade 3 in 4 patients (26.7%) in the NASH group; steatosis grade 1 was found in 33 patients (50.8%), steatosis grade 2 in 26 patients (40%), and steatosis grade 3 in 6 patients (9.2%) in the simple steatosis group. No significant relationship between the NLR and the steatosis grade in the USG was found for the NASH or the simple steatosis groups (p=0.60 and p=0.332, respectively).
When assessed in terms of the noninvasive fibrosis scores, APRI and FIB-4 scores in the NASH group were significantly higher than those of the simple steatosis and healthy control groups (p<0.001 and p=0.005, respectively).

In the ROC analyses conducted to distinguish the NASH patients, for APRI, the AUC was found to be 0.878 (95% confidence interval: 0.765–0.992); for the selected 0.324 cut-off value, the sensitivity was found as 80% and the specificity as 82.5%; for the NLR, the AUC was found to be 0.868 (95% confidence interval: 0.781–0.956), the sensitivity to be 86.5%, and the specificity to be 81% for the selected 1.793 NLR cut-off value (Figure 2). In the case of combined use of APRI and NLR, in the ROC curve for NLR whose cut-off value was determined as 0.324 for APRI; the AUC was found to be 0.856 (95% confidence interval: 0.689–1.0); for the 1.793 NLR cut-off value, the sensitivity was found to be 83.3%, and the specificity was found to be 90.9%. The calculated predictive value was found to be 90.9% and the negative predictive value was found to be 83.3%.

Discussion

The pathogenesis of NAFLD has not yet been fully elucidated, and it is thought to be multifactorial. Genetic factors, intestinal metabolic products, microbiota, various immunological mechanisms, and cytokines may play a role in pathogenesis. Obesity, DM, and metabolic syndrome are risk factors whose association with NASH has been proven in a number of studies. In our study, 76.4% of the patients in the NASH group had DM and 52.9% of them had hypertension. In addition, the BMI and waist-hip ratios of the patients in the NASH and simple steatosis groups were found to be significantly higher than those in the healthy control group. Waist-hip ratios were also higher in NASH patients than in simple steatosis patients.

Table 1. Demographic and clinical features in the patient and control groups

<table>
<thead>
<tr>
<th>Feature</th>
<th>NASH</th>
<th>Simple Steatosis</th>
<th>Control</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>51.0±9.6a</td>
<td>51.3±10.5a</td>
<td>46.3±15.5a</td>
<td>0.077</td>
</tr>
<tr>
<td>Female (n, %)</td>
<td>10. %66.7</td>
<td>39. %60</td>
<td>40. %61.5</td>
<td>0.891</td>
</tr>
<tr>
<td>NLR</td>
<td>2.16±0.49a</td>
<td>1.62±0.43a</td>
<td>1.51±0.31a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.66±7.70a</td>
<td>27.87±3.34a</td>
<td>23.64±2.23a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist-hip ratio</td>
<td>0.95±0.11a</td>
<td>0.89±0.54a</td>
<td>0.83±0.91a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leucocyte (10³/µL)</td>
<td>7.72±1.97a</td>
<td>7.34±1.90a</td>
<td>6.76±1.41a</td>
<td>0.055</td>
</tr>
<tr>
<td>Neutrophil (10³/µL)</td>
<td>4.75±1.40a</td>
<td>4.05±1.29a</td>
<td>3.60±0.89a</td>
<td>0.001</td>
</tr>
<tr>
<td>Lymphocyte (10³/µL)</td>
<td>2.24±0.59a</td>
<td>2.57±0.70a</td>
<td>2.42±0.56a</td>
<td>0.149</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>13.5±1.8a</td>
<td>14.5±1.4a</td>
<td>14.1±1.6a</td>
<td>0.082</td>
</tr>
<tr>
<td>Htc (%)</td>
<td>40.6±4.5a</td>
<td>42.3±5.5a</td>
<td>41.5±4.1a</td>
<td>0.357</td>
</tr>
<tr>
<td>RDW (%,cv)</td>
<td>14.3±1.5a</td>
<td>13.3±1.2a</td>
<td>13.1±0.8a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plt (10³/µL)</td>
<td>246.5±88.4a</td>
<td>272.6±57.3a</td>
<td>252.8±63.3a</td>
<td>0.138</td>
</tr>
<tr>
<td>MPV (fl)</td>
<td>10.3±0.9a</td>
<td>10.0±0.9a</td>
<td>10.3±0.9a</td>
<td>0.293</td>
</tr>
<tr>
<td>Fbg (mg/dL)</td>
<td>151.9±68.1a</td>
<td>101.7±12.1a</td>
<td>92.2±29.4a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>61.9±34.5a</td>
<td>20.9±5.7a</td>
<td>21.1±5.5a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>95.5±74.6a</td>
<td>26.1±12.7a</td>
<td>21.8±13.3a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ggt (U/L)</td>
<td>149.9±313.8a</td>
<td>33.8±35.1a</td>
<td>22.2±12.2a</td>
<td>0.041</td>
</tr>
<tr>
<td>Alp (U/L)</td>
<td>115.3±110.4a</td>
<td>86.0±35.9a</td>
<td>64.2±18.4a</td>
<td>0.060</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.0±0.7a</td>
<td>4.3±0.3a</td>
<td>4.4±0.3a</td>
<td>0.033</td>
</tr>
<tr>
<td>T. cholesterol (mg/dL)</td>
<td>213.3±68.6a</td>
<td>217.7±63.2a</td>
<td>188.8±31.4a</td>
<td>0.33</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>169.3±66.0a</td>
<td>177.1±96.4a</td>
<td>111.3±57.2a</td>
<td>0.047</td>
</tr>
<tr>
<td>Apri</td>
<td>0.77±0.56a</td>
<td>0.23±0.10a</td>
<td>0.25±0.09a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fibr-4</td>
<td>1.5±1.12a</td>
<td>0.87±0.44a</td>
<td>0.94±0.7a</td>
<td>0.005</td>
</tr>
</tbody>
</table>

NLR: neutrophil-lymphocyte ratio; BMI: body mass index; Hb: hemoglobin; Htc: hematocrit; RDW: red cell distribution width; Plt: platelet; MPV: mean platelet volum; FBG: fasting blood glucose; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyltransferase; ALP: alkaline phosphatase; T. cholesterol: total cholesterol; APRI: AST-to-platelet ratio index; FIB-4: fibrosis-4 score

Table 2. Liver biopsy findings in non-alcoholic steatohepatitis patients

<table>
<thead>
<tr>
<th>Histological finding</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degree of steatosis</td>
<td></td>
</tr>
<tr>
<td>5%-33%</td>
<td>8 (53.3)</td>
</tr>
<tr>
<td>33%-66%</td>
<td>5 (33.3)</td>
</tr>
<tr>
<td>&gt;66%</td>
<td>2 (13.4)</td>
</tr>
<tr>
<td>Inflammation</td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>8 (53.3)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>4 (26.7)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>3 (20)</td>
</tr>
<tr>
<td>Aneurism</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>5 (33.3)</td>
</tr>
<tr>
<td>Mild</td>
<td>4 (26.7)</td>
</tr>
<tr>
<td>Severe</td>
<td>6 (40.0)</td>
</tr>
<tr>
<td>Fibrosis</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>4 (26.7)</td>
</tr>
<tr>
<td>Mild</td>
<td>6 (40.0)</td>
</tr>
<tr>
<td>Moderate</td>
<td>1 (6.6)</td>
</tr>
<tr>
<td>Severe</td>
<td>4 (26.7)</td>
</tr>
</tbody>
</table>

When assessed in terms of the noninvasive fibrosis scores, APRI and FIB-4 scores in the NASH group were significantly higher than those of the simple steatosis and healthy control groups (p<0.001 and p=0.005, respectively).

In the ROC analyses conducted to distinguish the NASH patients, for APRI, the AUC was found to be 0.878 (95% confidence interval: 0.765–0.992); for the selected 0.324 cut-off value, the sensitivity was found as 80% and the specificity as 82.5%; for the NLR, the AUC was found to be 0.868 (95% confidence interval: 0.781–0.956), the sensitivity to be 86.5%, and the specificity to be 81% for the selected 1.793 NLR cut-off value (Figure 2). In the case of combined use of APRI and NLR, in the ROC curve for NLR whose cut-off value was determined as 0.324 for APRI; the AUC was found to be 0.856 (95% confidence interval: 0.689–1.0); for the 1.793 NLR cut-off value, the sensitivity was found to be 83.3%, and the specificity was found to be 90.9%. The calculated predictive value was found to be 90.9% and the negative predictive value was found to be 83.3%.

Discussion

The pathogenesis of NAFLD has not yet been fully elucidated, and it is thought to be multifactorial. Genetic factors, intestinal metabolic products, microbiota, various immunological mechanisms, and cytokines may play a role in pathogenesis. Obesity, DM, and metabolic syndrome are risk factors whose association with NASH has been proven in a number of studies. In our study, 76.4% of the patients in the NASH group had DM and 52.9% of them had hypertension. In addition, the BMI and waist-hip ratios of the patients in the NASH and simple steatosis groups were found to be significantly higher than those in the healthy control group. Waist-hip ratios were also higher in NASH patients than in simple steatosis patients.
In conclusion, the NLR is increased in NASH patients. This increase is probably due to inflammation in the liver and concomitant low-level systemic inflammation. Although it seems not appropriate to use the NLR solely as a marker alone in the diagnosis of NASH patients, we believe that its use, together with other diagnostic methods and scoring systems, would be useful in the management of patients with NAFLD and the selection of patients to be biopsied.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Necmettin Erbakan University Meram School of Medicine.

Informed Consent: Verbal informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.


Conflict of Interest: No conflict of interest was declared by the authors.

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References

2. Satapathy SK, Sanyal AJ. Epidemiology and natural history of nonalcoholic fatty liver disease. Semin Liver Dis. 2015; 35: 221-35. [CrossRef]
6. Meli R, Mattace Raso G, Calignano A. Role of innate immune respon-
se in non-alcoholic fatty liver disease: metabolic complications and
therapeutic tools. Front Immunol. 2014; 5: 177. [CrossRef]

7. Lu ZY, Shao Z, Li YL, Wulasihan M, Chen XH. Prevalence of and risk
factors for non-alcoholic fatty liver disease in a Chinese population:
[CrossRef]

8. Kekilli M, Tanoglu A, Sakin YS, Kurt M, Ocal S, Bagci S. Is the neut-
rophil to lymphocyte ratio associated with liver fibrosis in patients
[CrossRef]

9. Kumar R, Geuna E, Michalarea V, Guardascione M, Naumann U, Lo-
rente D et al. The neutrophil-lymphocyte ratio and its utilisation for
the management of cancer patients in early clinical trials. Br J Cancer
2015; 112: 1157-65. [CrossRef]

association between neutrophil/lymphocyte ratio and disease activity
in rheumatoid arthritis and anklyosing spondylitis. J Clin Lab Anal
2016; 30: 597-601. [CrossRef]

11. Day CP. Non-alcoholic steatohepatitis (NASH): where are we now and
where are we going? Gut 2002; 50: 585-8. [CrossRef]

al. Neutrophil-lymphocyte ratio as a predictor of disease severity in
ulcerative colitis J Clin Lab Anal 2013; 27: 72-6. [CrossRef]

Prognostic impact of platelet/lymphocyte and neutrophil/lymphocy-

neutrophil-to-lymphocyte ratio independently predicts survival in
patients with liver cirrhosis. Eur J Gastroenterol Hepatol 2013; 25:
435-41. [CrossRef]

Neutrophil-lymphocyte Ratio (NLR) could be better predictor than C-
reactive Protein (CRP) for liver fibrosis in Non-alcoholic Steatohepa-

insulin resistance in NASH: TNF-alpha or adiponectin? Hepatology
2004; 40: 46-54. [CrossRef]

TNF-α messenger ribonucleic acid (mRNA) in patients with nonalco-

18. Leite NC, Salles GF, Cardoso CR, Villela-Nogueira CA. Serum biomar-
kers in type 2 diabetic patients with non-alcoholic steatohepatitis and
advanced fibrosis. Hepatol Res 2013; 43: 508-15. [CrossRef]

cytokine and soluble cytokine receptor levels in patients with non-
alcoholic steatohepatitis. Liver Int 2006; 26: 39-45. [CrossRef]

20. Alkhouri N, Tamimi TA, Yerian L, Lopez R, Zein NN, Feldstein AE. The
inflamed liver and atherosclerosis: a link between histologic severity
of nonalcoholic fatty liver disease and increased cardiovascular risk.
Dig Dis Sci 2010; 55: 2644-50. [CrossRef]

invasive tests to predict liver fibrosis in chronic hepatitis B. Hepatol

22. Jayakumar S, Harrison SA, Loomba R. Noninvasive markers of fibrosis
and inflammation in nonalcoholic fatty liver disease. Curr Hepatology
Rep 2016; 15: 86-95. [CrossRef]