Predictive value of neutrophiltolymphocyte ratio in the severity of non-alcoholic fatty liver disease among type 2 diabetes patients

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Abstract

Background: **Non-alcoholic fatty liver disease is a progressive inflammatory disease that ultimately results in cirrhosis and liver failure. It is assosiciated with two step hit scenario; the first step is fat accumulationin liver and in the second step inflammation and fibrosis are the major compenents. The incidence of this disease is increasing worldwide, following rising incidences of obesity and diabetes mellitus.**

Aims: **The aim of this study is to analyze the relationship between non-alcoholic fatty liver disease andseverity and neutrophil-to-lymphocyte ratio among the patients having type 2 diabetes mellitus.**

Methods: **This study involved 143 patients with type 2 diabetes who were placed into four groups (grade 0, 1, 2, 3) based on steatosis level due to blinded ultrasonographic evaluation. Biochemical parameters and counts of total white blood cells, neutrophils, and lymphocytes were determined. Neutrophil-to-lymphocyte ratio was compared across the four patient groups.**

Results: **Levels of hemoglobin A1c, creatinine, alanine aminotransferase, high-density lipoprotein cholesterol and triglycerides were significantly different between the four patient groups (ANOVA p-values: p <0.001, p=0.011, p=0.002, p=0.034, p=0.002, respectively). Counts of white blood cells, neutrophils, lymphocytes, and neutrophil-to-lymphocyte ratio significantly differed between the groups (p <0.001). Neutrophil-to-lymphocyte ratio was positively correlated with steatosis grade (p < 0.001).**

Conclusions: **Neutrophil-to-lymphocyte ratio increases with increasing grade of non-alcoholic fatty liver disease in patients with type 2 diabetes, and may be a convenient marker to follow progression of non-alcoholic fatty liver disease.** (Acta gastroenterol. belg., **2016**, 79, **295-300**).

Key Words : diabetes mellitus, inflammation, Non-Alcoholic Fatty Liver Disease, steatosis.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most frequent cause of chronic liver disease worldwide, and its prevalence is likely related to the increasing incidences of obesity and insulin resistance (1). NAFLD affects 10-24% of people in certain countries, and is observed in up to 75% of patients with type 2 DM. Obesity, type 2 DM, hyperlipidemia, and hypertension frequently accompany NAFLD. Hepatic fibrosis occurs more frequently in obese patients with concurrent type 2 DM than in non-diabetic obese patients, which suggests that NAFLD is unrelated to obesity, and instead is related to type 2 diabetes and glucose intolerance (2,3).

NAFLD is a liver disease that begins with liver steatosis and progresses to advanced fibrosis and, ultimately, cirrhosis (4). Type 2 DM and NAFLD share several common pathophysiological characteristics, including increased oxidative stress, elevated hepatotoxic cytokines, and chronic inflammation (5). Liver steatosis generally results from extensive fat intake in diet, lipolysis in fatty tissues, fat re-synthesis and/or fat breakdown in hepatocytes, and low-density lipoprotein secretion. Accumulation of dense fat in the liver can result from increasing endoplasmic reticulum stress and oxidative stress (6,7).

Systemic inflammation is often measured by analyzing biochemical and hematological markers (8). A number of epidemiological studies have demonstrated that DM (9), metabolic syndrome (10), obesity (11), hypertension (12), and smoking habits (13) are related to low-grade chronic inflammation. Additionally, several studies have reported that inflammatory markers, including interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), white blood cell (WBC) count, and C-reactive protein (CRP), are associated with complications in diabetic patients (14,15,16,17). Moreover, it has recently been suggested that specific ratios of blood cells have significant prognostic value in cardiovascular diseases (18). Neutrophil-to-lymphocyte ratio (NLR) is a potential indicator of the inflammation in both cardiac and non-cardiac diseases (19), and several studies indicated that the presence and severity of complications in diabetic patients is related to NLR (20,21,22). In this study, we examined the associations of NLR to the incidence and steatosis level in patients with type 2 DM.

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Submission date : 01/04/2016
Acceptance date : 12/04/2016

Materials and Methods

Patients

This study enrolled 143 type 2 DM patients at the Internal Diseases clinic of Evliya Çelebi Training Research Hospital between July 2014 and February 2015. This study was approved by the Ethics Committee of Pamukkale University. Informed consent was obtained from all participants. The patients did not have insulin therapy. All patients had taken oral antidiabetics including sulfonylurea, metformin and dipeptidyl peptidase-4 (DPP4) inhibitor. Hemogram, serum total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, serum urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), and hemoglobin A1c (HbA1c) levels of all patients were measured and recorded. Patients were divided into four groups (grade 0, 1, 2, 3) based on hepatosteatosis levels determined by ultrasound. Patients who drink alcohol, have any active infection, chronic renal failure, chronic liver disease, congestive coronary failure, steroid use, or other malignancies were excluded from the study.

Evaluation of hepatosteatosis

Hepatosteatosis was evaluated with liver ultrasonography (Logiq 7, GE Medical System, Milwaukee, WI, USA), performed with a broadband curved-array transducer (4C, bandwidth, 1.5 to 4.5 MHz) . Radiologic evaluations were done blinded by experienced radiologists. In all cases we performed ultrasonography initially as a diagnosing method.

Evaluation of hepatosteatosis grade was performed through USG analysis as follows: grade 0: no fattening; grade 1 (cases with mild fattening): minimal diffuse increase in hepatic echogenicity; intrahepatic vein borders and diaphragm are observed clearly; grade 2 (cases with moderate fattening): moderate diffuse increase in hepatic echogenicity; appearances of intrahepatic veins and diaphragm mildly deteriorated; grade 3 (cases with advanced fattening): advanced diffuse increase in hepatic echogenicity. The echogenicity increase is at such a high level that it prevents visualization of intrahepatic veins and diaphragm (23). In order to avoid inter-personal differences, all USGs were performed by a single experienced radiologist.

Measurement of biochemical parameters

Whole blood samples were collected into 2.0 mL dipotassium (K2) ethylene diamine tetraacetic acid (EDTA) vacuum tubes (BD Vacutainer®, BD-Plymouth, UK). Total blood cell counts were performed using Coulter Gen-S automated hematology instruments (Beckman Coulter LH 780 Gen-S System; Miami, FL, USA; original reagents).

Venous blood samples were collected into an evacuated serum separator clot activator tube (Vacuette®, Greiner Bio-One Kremsmunster, Austria). Fresh blood samples were centrifuged at 1500 relative centrifugal force (rcf) for 15 min at room temperature within 1 hour of collection. Serum total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, serum urea, creatinine, AST, ALT, GGT, and ALP concentrations were measured on a Beckman Coulter AU680 analyzer (Beckman Coulter, Miami, FL, USA).

For HbA1C measurements, whole blood samples were collected into 2.0 mL dipotassium (K2) EDTA vacuum tubes (BD Vacutainer®, BD-Plymouth, UK). HbA1c measurements were immediately performed after blood collection, using fresh sample. HbA1cmeasurements were performed on a Tosoh G8 HPLC Analyzer (Tosoh Bioscience Inc., San Francisco, CA).

Statistical analysis

Statistical analyses were performed using GraphPad Prism version 6.05 (GraphPad Software, Inc., CA, USA). All data sets were tested for normality using Kolmogorov-Smirnov test. Data are expressed as means ± standard deviation (SD) for normally distributed values, or the median and interquartile range (IQR) for non-normally distributed values in study groups. Both parametric and non-parametric statistical tests were used where appropriate. The comparisons of data between groups were tested using one way ANOVA analysis of variance on ranks, and Tukey's test was used for the post hoc testing of normally distributed values in study groups. The comparisons of data between groups were tested using the Kruskal-Wallis analysis of variance on ranks, and Dunn's method was used for post hoc testing for non-normally distributed values in study groups. Correlation analyses were performed using Spearman's correlation analysis. P-values<0.05 were considered to be statistically significant.

Results

Characteristics of the four NAFLD patient groups, which represented the four grades of hepatosteatosis (grade 0, 1, 2, 3, represented by groups I, II, III, and IV, respectively), are given in Table 1. There was no significant difference in age or DM duration between the four groups ($p = 0.187$ and $p = 0.757$, respectively; Table 1).

Significant differences were observed for levels of HbA1c, creatinine, ALT, HDL cholesterol, and triglycerides between the study groups ($p < 0.001$, $p = 0.011$, $p = 0.002$, $p = 0.034$, $p = 0.002$, respectively; Table 2). HbA1c levels were significantly higher in group III and IV compared to group I ($p < 0.001$, $p < 0.01$, respectively; Table 2). Creatinine, HDL cholesterol levels, and ALT activities were significantly higher in group IV compared to group I ($p < 0.01$, $p < 0.05$, $p < 0.01$, respectively; Table 2). Triglyceride levels were

Table 1. — **Comparison of demographic data of patients between groups**

Variables	Group I (Grade 0) N=38	Group II (Grade 1) N=42	Group III (Grade 2) N=31	Group IV (Grade 3) N=32	Statistical Analysis P
Age (years)	51.5 ±14.2	51.6 ± 17.4	54.4 ± 9.9	57.9 ± 12.4	0.187
Sex (% female)	73.7	71.4	70.9	68.7	0.977
Duration of diabetes (years)	4.9 ± 2.4	5.3 ± 2.3	4.8 ± 2.5	4.9 ± 2.2	0.758

Data were presented as mean ± standart deviation (SD) for normally distributed values. Data were presented as median and interquartile ranges (IQR) for not normally distributed values. [a]P < 0.01, [b]P < 0.001, [c]P < 0.05 compared to group I; [d]P < 0.001 compared to group II; [e]P < 0.05 compared to group III. Data were tested using the one way ANOVA analysis of variance on ranks and Tukey test was used for post hoc testing for normally distributed values. *Data were tested using the Kruskal-Wallis Analysis of variance on ranks and Dunn's method was used for post hoc testing for not normally distributed values. A P value of less than 0.05 was considered as statistically significant.

significantly higher in group III and IV compared to group I (p <0.05, p <0.01, respectively; Table 2). No significant differences were observed for urea, AST, cholesterol, LDL cholesterol, ALP, and GGT levels between the study groups (Table 2).

Significant differences in WBC, hematocrite (HTC), neutrophil, lymphocyte, and NLR levels were observed between the study groups (p <0.001, p = 0.004, p <0.001, p <0.001, p <0.001, respectively; Table 3). WBC, neutrophil and NLR levels were significantly higher in group II (p <0.01, p <0.05, p <0.05, respectively; Table 3), group III (p <0.01, p <0.01, p <0.01, respectively; Table 3), and group IV (p <0.001, p <0.001, p <0.001, respectively; Table 3) compared to group I. In addition, neutrophil and NLR levels were higher in group IV compared to group II (p <0.001, p <0.001, respectively; Table 3), and neutrophil levels were higher in group IV compared to group III (p <0.05; Table 3). HTC and lymphocyte levels were higher in group III (p <0.05, p <0.01, respectively; Table 3), and IV (p <0.01, p <0.01, respectively; Table 3) compared to group I. In addition, lymphocyte levels were higher in group IV compared to group II (p <0.001; Table 3). No significant differences were found inRed blood cell distribution width (RDW), Platelet (PLT), and mean platelet volume (MPV) levels between the study groups (Table 3).

A significant positive correlation was found between NLR and WBC, (p <0.001, r = 0.440).−Additionally, a significant positive correlation was found between NLR and HbA1c, creatinine, and triglyceride levels (p = 0.005, r = 0.334; p <0.001, r = 0.043; p = 0.017, r = 0.228, respectively). A significant negative correlation was found between NLR and HDL cholesterol levels (p = 0.110, r = -0.154). In addition, a positive correlation was observed between NLR and steatosis grade (p <0.001, r = 0.544) (Figure 1).

Discussion

NAFLD pathogenesis is described by a 2-step model. The first step is fat accumulation in the liver including cholesterol and trigliseride, second step is associated with insulin resistance (24,25). Oxidative stress, mitochondrial disorders, inflammatory cytokines (such as TNFα), and hormones (such as adiponectin and leptin) are responsible for the second step, which leads to liver inflammation and fibrosis (26). Many cytokines that are induced by endotoxins are factors that cause liver damage in NAFLD. For example, TNFα is increased in the serum of patients with NAFLD. Increase in TNFα can contribute to accumulation of reactive oxygen species (ROS); long-term exposure to ROS and inflammation can contribute to hepatocyte death (27).

There are epidemiological studies reporting the relationship between NAFLD prevalence and inflammatory indicators, including WBC count, high sensitive C-reactive protein (hs-CRP), IL-6, IL-8 and TNFα (28,29,30,31,32). It was reported that there is a positive correlation between grade of steatosis and WBC count in patients diagnosed with NAFLD (28). Additionally, it has been independently reported that hs-CRP levels are elevated in the presence of liver steatosis (29), and that hs-CRP levels correlate positively with grade of liver steatosis in patients with NAFLD (30). Moreover, it has been reported that there is a significant relationship between hs-CRP levels and

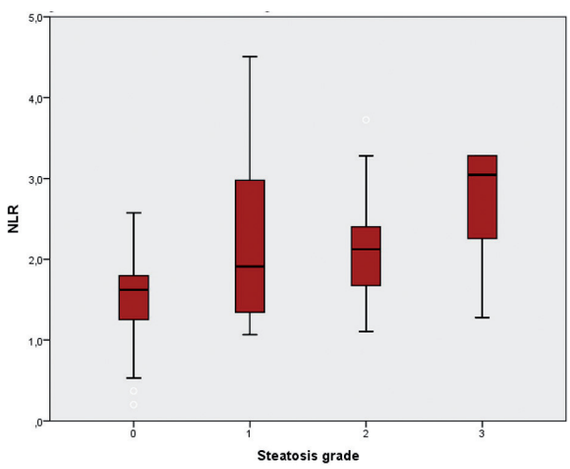

Fig. 1. — NLR values in the steatosis grade. NLR: Neurophil to-lymphocyte ratio

Table 2. — **Comparison of the values of biochemical parameters between groups**

	Group I (Grade 0) N=38	Group II (Grade 1) N=42	Group III (Grade 2) N=31	Group IV (Grade 3) N=32	Statistical Analysis P
HbA1C (%)	5.4 (4.8-5.6)	5.8 (5.5-7.0)	7.3 (6.3-8.5)[b]	6.5 (5.7-7.3)[a]	< 0.001*
Urea (mg/dl)	27.0 (22.0-39.0)	30.0 (21.5-40.5)	34.5 (28.0-41.0)	33.0 (25.0-46.0)	0.466
Creatinine (mg/dl)	0.7 (0.6-0.8)	0.8 (0.6-0.9)	0.7 (0.7-0.9)	0.9 (0.7-1.1)[a]	0.011*
AST (U/L)	19.0 (15.0-25.0)	19.0 (15.0-29.0)	20.0 (17.0-28.0)	20.0 (18.0-25.0)	0.604
ALT (U/L)	14.0 (9.5-21.5)	19.0 (12.0-29.0)	19.0 (15.0-31.0)	25.0 (20.0-35.0)[a]	0.002*
Cholesterol (mg/dl)	190.7 ± 58.8	199.1 ± 40.4	197.5 ± 56.8	212.8 ± 32.9	0.425
LDL-chol (mg/dl)	112.7 ± 47.5	118.6 ± 36.5	111.1 ± 42.9	129.3 ± 28.7	0.353
HDL-chol (mg/dl)	54.5 ± 20.8	47.3 ± 12.5	43.1 ± 13.7	43.0 ± 15.0[c]	0.034*
Triglyceride (mg/dl)	106.0 (78.5-145.8)	153.5 (108.8-194.0)	154.0 (95.8-241.0)[c]	182.0 (123.0-228.5)[a]	0.002*
ALP (U/L)	79.2 ± 26.3	78.3 ± 28.3	99.8 ± 55.5	87.8 ± 26.3	0.150
GGT (U/L)	17.0 (13.0-37.8)	26.5 (17.0-41.5)	36.0 (16.0-71.0)	27.5 (19.8-41.0)	0.122

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, GGT: Gamma-glutamyl transferase. Data were presented as mean ± standart deviation (SD) for normally distributed values. Data were presented as median and interquartile ranges (IQR) for not normally distributed values. [a]P < 0.01, [b]P < 0.001, [c]P < 0.05 compared to group I; [d]P < 0.001 compared to group II; [e]P < 0.05 compared to group III. Data were tested using the one way ANOVA analysis of variance on ranks and Tukey test was used for post hoc testing for normally distributed values. *Data were tested using the Kruskal-Wallis analysis of variance on ranks and Dunn's method was used for post hoc testing for not normally distributed values. A P value of less than 0.05 was considered as statistically significant.

Table 3. — **Comparison of the values of blood cell count parameters between groups**

	Group I (Grade 0) N=38	Group II (Grade 1) N=42	Group III (Grade 2) N=31	Group IV (Grade 3) N=32	Statistical Analysis P
WBC	6.3 ± 2.2	7.7 ± 2.1[a]	8.0 ± 1.7[a]	8.9 ± 1.9[b]	< 0.001*
HTC (%)	37.5 ± 6.8	40.4 ± 4.9	41.5 ± 5.7[c]	42.3 ± 5.6[a]	0.004*
RDW	14.5 (13.5-17.0)	14.0 (13.4-14.9)	13.9 (13.3-14.7)	13.9 (13.2-15.4)	0.367
PLT	240.7 ± 101.0	255.6 ± 64.6	266.3 ± 82.8	261.7 ± 76.8	0.582
Neutrophil	56.6 (48.0-58.4)	59.2 (51.0-68.3)[c]	62.0 (56.4-65.1)[a]	68.6 (61.5-77.8)[bdc]	< 0.001*
Lenfocyte	33.6 (31.5-37.4)	30.8 (22.2-38.5)	28.8 (25.1-31.0)[a]	22.3 (15.9-27.2)[ad]	< 0.001*
Neutrophil / lenfocyte ratio	1.6 (1.3-1.8)	1.9 (1.3-3.1)[c]	2.2 (1.8-2.5)[a]	3.1 (2.3-5.0)[bd]	< 0.001*
MPV	8.7 ± 1.1	8.4 ± 0.7	8.7 ± 1.1	8.6 ± 0.9	0.575

WBC: White blood cell, HTC: Hematocrite, RDW: Red blood cell distribution width, PLT: Platelet, MPV: Mean platelet volüme. Data were presented as mean ± standart deviation (SD) for normally distributed values. Data were presented as median and interquartile ranges (IQR) for not normally distributed values. [a]P < 0.01, [b]P < 0.001, [c]P < 0.05 compared to group I; [d]P < 0.001 compared to group II; [e]P < 0.05 compared to group III. Data were tested using the one way ANOVA analysis of variance on ranks and Tukey test was used for post hoc testing for normally distributed values. *Data were tested using the Kruskal-Wallis analysis of variance on ranks and Dunn's method was used for post hoc testing for not normally distributed values. A P value of less than 0.05 was considered as statistically significant.

risk of cardiovascular disease. Although hs-CRP, total cholesterol, TG, and LDL were found to be higher in NAFLD patients than in a control group, HDL levels of NAFLD patients were found to be lower (30). Our study corroborates these findings, as a positive correlation between NAFLD grade and WBC count and TG levels and a negative correlation between NAFLD grade and HDL levels were determined in our study. Increased levels of hepatic and circulating IL-6were reported in patients with non-alcoholic steatohepatitis (NASH) in comparison to patients with steatosis and to a control population (31). Additionally, it was reported that serum levels of IL-8 and TNFα of NASH patients are higher than in a control group (32).

Several studies have demonstrated the importance of NLR as an indicator of subclinical inflammation in coronary artery disease (33,34,35). Indeed, it was reported that there is a direct relationship between NLR and the prevalence and severity of coronary artery disease (36). Furthermore, it has been reported

that the presence of NAFLD is an independent risk factor for the development of cardiovascular disease in patients with type 2 DM (37). A study regarding the relationship between liver fattening, fibrosis grade, and NLR reported that NLR levels increase as the severity of inflammation and fibrosis increases in patients with NAFLD (38). Likewise, a strong relationship between NAFLD presence and increased NLR was detected in our study. Moreover, WBC count, neutrophil, and lymphocyte counts were found to be related to NAFLD grade in our study. This highlights the novel element of our study: namely that NLR increases along with the presence and steatosis level, which indicates the utility of NLR as a practical and non-invasive indicator of NAFLD progression.

Our study shows that NLR increases in association with increased steatosis level in patients with type 2 DM. These data suggest that NLR may be useful as a convenient and non-invasive marker to follow NAFLD disease progression. These results indicate that more extensive and prospective studies are warranted to study the use of NLR as a non-invasive test to monitor NAFLD disease progression.

In a recent study via Singh et al (39) investigated that, there are many imaging techniques likewise; elastography, computed tomography, and magnetic resonance imaging to estimate hepatic fat content both in diabetic and non diabetic patients including metabolic syndrome. In our manuscript we only performed imaging in diabetic patients. However, further studies are needed to support hepatosteatosis level in non diabetics.

Limitations

Although liver biopsy is the gold-standard method for determining hepatosteatosis, as a result of ethical considerations and a large body of research advocating the use of USG to determine NAFLD, liver biopsy is routinely avoided to evaluate the presence of NAFLD. On the other hand, USG is relatively sensitive in detecting hepatosteatosis, with a sensitivity of 89% and a specificity of 93% (40); USG was the method of choice for this present study. The number of patients in this study was limited, and stratification of patients based on obesity status was not considered; nor were medications used by the patients examined in detail. These factors may to some extent diminish the significance of these findings. Additionally, another limitation is that the study had a cross-sectional design. Future prospective studies may strengthen the significance of these current findings.

Disclosure

The authors have no financial interestsdo disclose regarding any of the products or methods used in this study.

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