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Correlation between red cell distribution width and grade of hepatic steatosis and liver enzymes in patients with Non-Alcoholic steatohepatitis

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Abstract

Red cell distribution width (RDW) is a marker of chronic inflammation. Hepatic diseases are often associated with several hematological complications; therefore, high levels of RDW are seen in hepatic diseases. Given the fact that factors which cause non-alcoholic steatohepatitis such as inflammation, oxidative stress, free oxygen radicals, and inflammatory cytokines may have a role in the discrepancy in the erythrocyte volume, we aimed to investigate the relationship between RDW and hepatic enzymes and Grade of hepatic steatosis. A total of 120 patients with non-alcoholic steatohepatitis using abdominal ultrasonography which was performed by a gastroenterology specialist, were included in the study. Biochemical analysis, complete blood count, other blood tests were performed. Spearman's Rho correlation test was used to analyze the relationship between the parameters and p value was accepted significant if it's <0.05. As a result of our study, there was no significant relationship between RDW and liver steatosis degree (p: 0.704). Also, significant relationship was not found between RDW and alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) and lactate dehydrogenase (LDH) (p values were 0,223, 0,856, 0,318, 0,597, 0,195 consecutively). In our study, we tried to find an answer to if there is a relationship between RDW alterations and inflammation which takes place in the pathogenesis of non-alcoholic steatohepatitis. As a result, we found no significant correlation between the RDW values and the degree of steatosis of liver and also liver enzymes.

Keywords: Red cell distribution width, non-alcoholic steatohepatitis, liver enzymes

Introduction

As most of the non-alcoholic steatohepatitis (NASH) which do not manifest with the findings of hepatitis, a new definition, non-alcoholic fatty liver disease (NAFLD) have been given prominence and NASH has been started to be evaluated under this concept [1]. Today, NAFLD is a preferred term and it represents a wide clinical spectrum from simple steatosis to steatohepatitis, advanced fibrosis, and cirrhosis [1].

The component which is necessary for the development of NAFLD is inflammation [2]. The hypothesis of multiple strikes is used in the explanation of the pathogenesis of NAFLD [3].

The main pathophysiological event in NASH is insulin resistance [4]. It is well-established that insulin resistance, oxidative stress, inflammation, inflammatory cytokines have important role in the pathogenesis and progression of NASH. The main criterion for the diagnosis of NASH is the presence of inflammation [4]. Tumor necrosis factor-alpha (TNF-α), which is released from adipose tissues, causes proinflammatory cytokine release [2].

Ultrasonography (USG) is the initially method in patients with the suspicion of steatohepatitis, thanks to its easy-to-use, cost-efficient, and non-invasive practice which involves no radiation [5]. It also enables grading of steatosis. It has been demonstrated that overall sensitivity and specificity of USG is about 80 to 95% and 90 to 95%, respectively in the diagnosis of steatohepatitis.

RDW is a statistical parameter derived from erythrocyte histograms and it can be measured in routine blood count without any additional cost [6]. It demonstrates the distribution width according to the sizes of the erythrocytes. It is a quantitative measure of the volume difference of erythrocytes. In other words, it is an objective marker of anisocytosis. There are also data in the literature suggesting that RDW is a marker of chronic inflammation [7,8].

Hepatic diseases are associated with several hematological complications [9]. Decreased erythropoiesis, decreased life cycle of erythrocytes, erythrocyte production defect, hemolysis, findings of bone marrow suppression, such as decreased bone marrow cellularity and vacuolization, in case of splenomegaly with splenic sequestration, erythrocyte membrane defect and erythrocyte

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maturation defects, thereby, leading to elevated RDW levels are among the main pathogenetic factors [7,10].

Given the fact that factors which cause non-alcoholic steatohepatitis such as inflammation, oxidative stress, free oxygen radicals, and inflammatory cytokines may have a role in the discrepancy in the erythrocyte volume, we aimed to investigate the relationship between RDW and hepatic enzymes and Grade of hepatic steatosis.

Material and Methods

The study protocol was approved by the institutional Ethics Committee. A written informed consent was obtained from each patient. The study was conducted in accordance with the principles of the Declaration of Helsinki.

A total of 120 patients with non-alcoholic steatohepatitis using abdominal ultrasonography which was performed by a gastroenterology specialist between December 2011 and December 2012 were included in the study. Patients with other hepatic diseases as assessed by clinical and laboratory examinations were excluded from the study.

None of the patients included in the study had a history of alcohol use or chronic liver disease. Chronic liver diseases, hepatic tumors and cysts, storage diseases of the liver such as hemochromatosis and Wilson's disease and conditions related to anemia were excluded using

laboratory and imaging methods. HbsAg, anti-HBs, anti-HBcIgM, and anti-HCV markers were examined to exclude viral hepatitis. Anti-mitochondrial antibody, anti-smooth muscle antibody, anti-nuclear antibody, and anti-liver kidney antibody were measured to exclude autoimmune hepatitis. End-stage liver failure such as cirrhosis was also excluded with the absence of laboratory findings such as low levels of albumin, prolonged prothrombin time, and hyperbilirubinemia and other findings of ascites and portal hypertension on physical examination findings and imaging methods.

Anemia was excluded by the measurement of complete blood count, iron, iron binding capacity, ferritin, and vitamin B12. A history of drug use causing hepatic steatosis was excluded. Pregnant women were also excluded from the study. Some of the patients with diabetes mellitus who were on oral antidiabetic drugs alone were included.

Complete blood count, fasting blood glucose, fasting insulin, total cholesterol, triglyceride and other cholesterol parameters, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), albumin, bilirubin, prothrombin time, and thyroid function tests and other blood parameters were measured. Complete blood count was measured using the hemoglobin spectrophotometry method and cell impedance method via Beckman Coulter LH 780 blood count device. The RDW is calculated by division of histogram width of 68.26% of erythrocytes in one standard deviation to MCV and, then, multiplication with 100. The device automatically calculates RDW and mean corpuscular volume (MCV). The normal range for RDW was accepted between 11.8 and 14.3%. Insulin was measured using chemiluminescence method through Siemens Immulite 2000 device. Glucose, blood lipids, and liver enzymes were measured using spectrophotometry through Architect C 16000 device.

The body mass index (BMI) was calculated in all patients by dividing body weight by height squared (kg/m2). A BMI of 18.5 to 24.9 kg/m2 was accepted normal, of 25 to 29.9 kg/m2 was accepted overweight, of 30 to 39.5 kg/m2 was accepted obese, and of >40 kg/m2 was accepted morbid obese.

A waist circumference of <94 cm in men and of <80 cm in women was accepted normal, of 94 to 102 cm in men and 80 to 88 cm in women was accepted risky, and of >102 cm in men and >88 cm in women was accepted high-risk.

Homeostasis model assessment insulin resistance (HOMA-IR) was calculated by the following formula: fasting plasma glucose mg/dL x fasting insulin level µU/mL/405. A HOMA-IR value above 2.5 indicates insulin resistance. The USG examination was performed by a gastroenterologist. The degree of steatosis was graded as Grade I, Grade II, and Grade III.

Statistical Analysis

Statistical analysis was performed using the SPSS version 17.0 software (SPSS Inc., Chicago, IL, USA). Descriptive data were expressed in mean and standard deviation (SD). The Spearman's rho correlation test, independent sample t-test, one way variance of analysis (ANOVA), and Mann-Whitney U test were used to analyze data. A p value of <0.05 was considered statistically significant.

Results

Of 120 patients, 63 (52.5%) were females and 57 (47.5%) were males. The mean age was 47.52±11.32 (range: 19 to 85) years. The mean BMI was 31.24±6.28 (range: 23,6 to 41,82) kg/m2 with a mean BMI of 29.20±4.41 kg/m2 in females and of 33.14±6.64 kg/m2 in males. The mean waist circumference was 104.10±15.41 (range: 76 to 136) cm with a mean waist circumference of 93.69±15.73 cm in females and of 114.12±14.97 cm in males. The mean RDW was 14.06±1.34 (range: 12,3 to 19,6)% and the mean hemoglobin was 14.62±1.13 (range: 13,0 to 17,7) g/dL. The mean cell volume (MCV) value was 87.54±4.39 (range: 80 to 103) fL. The mean AST value was 42.99±28.8 (range: 13 to 205) U/L, the mean ALT value was 66.05±43.56 (range: 10 to 255) U/L, the mean ALP value was 81.15±24.39 (range: 30 to 153) U/L, the mean GGT value was 54.75±40.22 (range: 9 to 224) U/L, the mean LDH value was 212.98±57.78 (range: 14 to 416) U/L, the mean fasting blood glucose level was 107.42±26.13 (range: 75 to 218) mg/dL, the mean fasting insulin value was 15.64±11.77 µIU/mL (range : 9.3 to 29.3), the mean HOMA-IR value was 4.27±3.41 (range: 0,35 to 19,60), and the mean triglyceride value was 195.70±124.24 (range: 46 to 1134) mg/dL (Table 1).

The patients with hepatic steatosis (n=120) were classified as Grade 1 (=1), Grade 2 (=2), and Grade 3 (=3) according to the USG findings. The mean value according to the USG images was calculated as 1.92±0.57. Grade 1 steatosis was detected in 25 patients (20.8%), Grade 2 steatosis in 80 patients (66.7%), and Grade 3 steatosis in 15 patients (12.5%) (Table 2).

Table 1. Sociodemographic characteristics, laboratory values, and reference ranges

Variable	Mean value	Min-Max	Reference range and unit
Age (year)	47.52	19-85	-
Sex (n; F/M)	n=120; 63/57	-	-
BMI	31.24+6.28	23.60-41.82	kg/m2
Waist circumfer-ence	104.10+15.41	76-136	cm
RDW	14.06+1.34	12.3-19.6	11.8- 14.3 %
Hemoglobin	14.62+1.13	13.0-17.7	13.6-17.2 gr/dL
MCV	87.54+4.39	80-103	80.7-95.5 fL
Platelet	268.47+68.60	89-439	(156-373)X 103/mL
AST	42.99+28.28	13-205	5-34 U/L
ALT	66.05+43.56	10-255	0-55 U/L
ALP	81.15+24.39	30-153	40-150 U/L
GGT	54.75+40.22	9-224	9-64 U/L
LDH	212.98+57.78	14-416	125-243 U/L
Triglyceride	195.70+124.24	46-1134	0-149 mg/dL
Indirect bilirubin	0.54+ 0.19	0.4-1.2	0.2-0.7 mg/dl
Direct bilirubin	0.32+0.12	0.2-0.8	0-0.5 mg/dl
Total protein	7.16+1.52	5.2-10.0	6-8.3 gr/dl
Albumin	4.32+0.62	2.8-6.4	3-4.5 gr/dl
Prothrombin time	11.20+1.82	8-20	10-14 sn
Fasting glucose	107.42+26.13	75-218	70-105 mg/dL
HOMA-IR	4.27+3.41	0.35-19.60	<2.5

Table 2. Comparison of socidemographic characteristics and laboratory values according to the degree of steatosis

Variable	Grade 1 Mean + SD	Grade 2 Mean + SD	Grade 3 Mean + SD	p
Age (year)	48.20+14.74	47.84+10.39	44.67+9.89	0.036
Sex (n; F/M)	n=25; 13/12	n=80; 41/39	n=15; 9/6	0.692
Fasting glucose	111.04+8.85	105.89+27.56	109.53+14.56	0.721
HOMA-IR	4.41+3.61	3.83+2.69	6.39+5.44	0.187
RDW	13.96+0.95	14.02+1.41	14.42+1.55	0.704
Hemoglobin	14.69+1.30	14.62+1.02	14.46+1.41	0.548
MCV	87.46+3.93	87.57+4.46	87.56+5.01	0.930
Platelet	269.64+61.80	262.10+69.05	300.47+71.42	0.296
AST	34.92+25.21	45.73+30.91	41.87+12.58	0.298
ALT	49.36+36.57	69.21+46.78	77.00+28.36	0.032
ALP	72.56+18.69	83.56+26.19	82.60+20.47	0.122
GGT	40.80+43.16	55.20+37.01	75.60+44.85	0.008
LDH	203.84+36.91	215.98+63.99	212.20+52.30	0.551
Triglyceride	168.44+65.17	201.60+139.94	209.73+108.10	0.253

Laboratory and USG findings of all patients were statistically analyzed. There was a relationship between RDW and Grade of steatosis (p=0.704, r=0.035), between RDW and AST (p=0.856, r=-0.017), between RDW and ALT (p=0.223, r=-0.112), between RDW and ALP (p=0.318, r=0.092), between RDW and GGT (p=0.597, r=0.049), and between RDW and LDH (p=0.195, r=0.119) (Table 3). However, there was no statistically significant relationship between RDW and Grade of hepatic steatosis and liver enzymes.

Table 3. Correlation coefficients and significance level between RDW and associated variables

	USG	AST	ALT	ALP	GGT	LDH
RDW	r:0.035	r:-0.017	r:-0.112	r:0.092	r:0.049	r:0.119
	p:0.704	p:0.856	p:0.223	p:0.318	p:0.597	p:0.195

Discussion

The component which is necessary for the development of NAFLD is inflammation [2]. The RDW is a marker of disturbance developing during differentiation and maturation of erythrocytes due to chronic inflammation and oxidative stress [11]. There are data in the literature suggesting that RDW is a marker of chronic inflammation and RDW increases with inflammation [7,8].

The relationship between RDW and inflammation in hepatic diseases has been extensively studied in several studies. In the study of Sandra Milic et al. [12], 241 patients with alcoholic and non-alcoholic hepatic cirrhosis and anemia (hemoglobin< 13 g/dL in males and hemoglobin <12 g/dL in females) were retrospectively analyzed. The mean RDW was found to be higher in both patient groups, although it did not reach statistical significance (p>0.05). In a study of Hu Z. et al. [13], 298 patients with hepatic diseases were retrospectively evaluated. The aforementioned study included the patients with chronic hepatitis b (HBV) without cirrhosis, those with cirrhosis developing after HBV, those with recent diagnosis of hepatocellular cancer, those with primary biliary cirrhosis, and those with alcoholic hepatic cirrhosis. A statistically significant increase was found in the RDW at the end of the study. Furthermore, there was a positive correlation between the severity of Child Pugh score and RDW in patients with cirrhosis. The authors concluded that RDW was a potential prognostic marker of hepatic disease. Interestingly, both aforementioned studies have in common in terms of the presence of a positive correlation between the RDW and inflammation in patients with chronic liver disease.

Obesity is associated with inflammation and RDW increases in inflammatory conditions [4,14]. Accordingly, in the study of Fujita B et al. [15], diet-induced obesity was used in an animal model for 15 weeks using 79 adult male mice aged between 13 and 17 which were normal and overweight, and the relationship between obesity and RDW was analyzed. The authors found a positive correlation between the RDW and obesity in overweight animals (p=0.015) and increased RDW in animals in which diet was modified.

Furthermore, the relationship between RDW and inflammation has been investigated in many diseases rather than liver diseases. The common finding of previous studies is that these studies support the opinion that inflammatory processes lead to the increase in RDW [8]. In the studies conducted in diseases such as inflammatory bowel diseases [16], coronary artery diseases [17], heart failure [18], renal failure [19], mortality increasing with aging [20], and breast cancer [21], high RDW levels were found. In some of these studies, increased RDW values proportional to the severity of disease were reported and it was suggested that RDW could be used as an inflammatory marker as C-reactive protein and erythrocyte sedimentation rate [17]. But in our study, there was no correlation found between RDW and inflammation.

In previous studies, although the relationship between inflammation and RDW has been investigated, a positive correlation was unable to be found. In the study of Kurt OK et al. [22], which included 98 patients with obstructive sleep apnea syndrome (OSAS), the relationship between OSAS and RDW was investigated and OSAS was found to be a risk factor for cardiovascular diseases. In another study, activated sympathetic nervous system, oxidative stress, vascular endothelial dysfunction, metabolic dysregulation, hypercoagulability, and inflammation were all found to be effective in pathogenesis of OSAS and heart diseases [23]. However, the authors found no relationship between the OSAS and RDW. And also, no correlation was found between inflammation and RDW alterations in our study.

While there was a positive correlation between inflammation and RDW in some studies, in some no increase was found between RDW whit inflammation.

Given the fact that inflammation increases the RDW, in the present study, we investigated whether inflammation, which plays a role in the pathogenesis of NAFLD, altered the RDW values. Based on our study findings, we found no significant correlation between the RDW and hepatic steatosis and liver enzymes.

The cause of this may be the limitation of cases conducted in our study. And also, while diagnosing steatohepatitis, we performed ultrasound technique which is more simple and subjective than liver biopsy which is an invasive procedure. This can be the another cause of unsignificant result of our study.

Conclusion

In conclusion, our study results show no significant relationship between RDW and Grade of hepatic steatosis and liver enzymes. However, further, large-scale studies are needed to confirm these findings and to establish a definite conclusion.

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