**Name of journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO: 4026**

**Columns:** **BRIEF ARTICLE**

**Is increased red cell distribution width an indicating marker of nonalcoholic steatohepatitisand fibrotic stage?**

Cengiz M *et al.* Non-alcoholic steatohepatitis and fibrosis

Mustafa Cengiz, Burcu Aslan Candır, Güldal Yılmaz, Gülen Akyol, Seren Ozenirler

**Mustafa Cengiz, Seren Ozenirler,** Department of Gastroenterology, Gazi University Faculty of Medicine, 06500 Ankara, Turkey

**Burcu Aslan Candır**, Department of Internal Medicine, Gazi University Faculty of Medicine, 06500 Ankara, Turkey

**Güldal Yılmaz, Gülen Akyol**, Department of Pathology, Gazi University Faculty of Medicine, 06500 Ankara, Turkey

**Authors contributions**: Cengiz M and Ozenirler S;designed the study, contributed the acquisition of data, analysis and interpretation of data; Cengiz M performed the statistical analyses, drafting the article and revising it critically for important intellectual content; Candır BA recruited the patients, contributed the interpretation of data; Yılmaz G and Akyol G evaluated the liver histopathology, analysed the data and contributed the interpretation of data.

**Correspondence to:** **Mustafa Cengiz, MD,** Gastroenterology Division, University of Gazi, Besevler, 06500 Ankara, Turkey. drmustafacen@gmail.com

**Telephone:** +90-312-20227586 **Fax:** +90-312-2213202

**Received:**  June 9, 2013 **Revised:** August 19, 2013

**Accepted:** September 15, 2013

**Published online:**

**Abstract**

**AIM**: To evaluate the indicating property of red cell distribution width (RDW) in the presence of non-alcoholic steatohepatitis (NASH) and the association between RDW and fibrotic scores.

**METHODS**: A retrospective study including sixty two biopsy proven NASH, 32 simple steatosis patients and 30 healthy controls was carried out. Control group was created from healthy individuals.The correlation between the clinical and histopathological features of NASH patients and RDW values was evaluated. The liver fibrosis scores were measured using a 0 to 4 point scale and then were divided in to two groups; fibrosis scores 0-1 as mild and fibrosis scores 2-4 were grouped as advanced fibrosis. RDW values were compared between NASH, simple steatosis and healthy controls. Univarite and multivariate analyses were performed for evaluating the independent predicting factors for the presence of liver fibrosis due NASH.

**RESULTS**: Patients with NASH had higher RDW values compared with simple steatosis and healthy control groups 14.28 ± 0.25 *vs* 13.37 ± 0.12, 12.96 ± 0.14 (*P* < 0.01), respectively. Patients with advanced fibrosis had higher RDW values than mild fibrosis group 15.86 ± 0.4 *vs* 13.63 ± 0.67, *P*<0.01 respectively**.** RDW was also correlated with fibrotic scores *r* = 0.579 and *P* < 0.01. The variables those were significant in the univariate analysis were evaluated in multivariate logistic regression analysis and RDW was an independent predicting factor of NASH (OR = 1.75, 95%CI: 1.129-2.711, *P* < 0.05).

**CONCLUSION**: RDW a new non-invasive marker can be used to demonstrate the presence of NASH and indicate the advanced fibrotic scores.

© 2013 Baishideng. All rights reserved.

**Key words**: Non-alcoholic steatohepatitis; liver fibrosis; Red cell distribution width; simple steatosis; non-invasive marker; liver biopsy

**Core tip:** We evaluated the role of red cell distribution width (RDW) in the indicating nonalcoholic steatohepatitis by comparing the values of biopsy proven non-alcoholic steatohepatitis (NASH) patients with simple steatosis and healthy controls. The independent predictors of the presence of NASH and advanced liver fibrosis were evaluated by using multivariate logistic regression analyses and RDW was statistically significant independent predictor.

Cengiz M, Candır BA, Yılmaz G, Akyol G, Ozenirler S. Is increased red cell distribution width an indicating marker of nonalcoholic steatohepatitis and fibrotic stage?

**Available from:**

**DOI:**

**INTRODUCTION**

Nonalcoholic steatohepatitis (NASH) is a part of the spectrum of non-alcoholic fatty liver disease (NAFLD) which is consisted of simple fatty liver, NASH, associated advanced fibrosis and cirrhosis[[1](#_ENREF_1)]. NAFLD became one of the most common form of chronic liver diseases in the United States and all over the world with the prevalence of 10%-24% of the population[[2-5](#_ENREF_2)]. NASH is characterized by ballooning, degeneration, and lobular inflammation with various stages of fibrosis. Lobular inflammation is a hallmark of NASH which is characterized by infiltration of lymphocytes, mononuclear cells and polymorphonuclear neutrophils. Steatosis is present in all cases and affecting hepatic lobules[[6](#_ENREF_6)]. Simple steatosis is a benign condition with minimum progression, despite a high rate of progression to cirrhosis in 15%–25% of patients with NASH[[7](#_ENREF_7)]. Liver fibrosis is a result of chronic injury[[8](#_ENREF_8)], and in the absence of approved treatment modalities for NASH, care should be taken on the detection of advanced fibrosis. A liver biopsy is the only method for distinguishing NASH from other diagnoses and evaluating the damage and fibrosis of liver however it’s an invasive and expensive procedure with critical important complications such as bleeding and perforation. Therefore, it has been attempted to find noninvasive methods for detecting the presence of NASH, liver fibrosis and avoiding these procedure related complications.

Chronic inflammation plays a significant role in disease progression to NASH[[9](#_ENREF_9)] and also in some studies it has been shown that some inflammatory cytokines were higher in patients with NASH[[10](#_ENREF_10), [11](#_ENREF_11)].

Red cell distribution width (RDW) is a parameter of variation of circulating red cells. This parameter demonstrates the heterogeneity of red cell volume and is a component of the complete blood count (CBC).The RDW is also widely available, inexpensive and easily repeatable marker that measures red blood cell (RBC) volume variability. Recent reports have demonstrated that elevated RDW values were related to negative outcomes in cardiovascular and metabolic disorders, colon cancer and stroke independent of hemoglobin (HGB) values[[12-16](#_ENREF_12)]. The association between the RDW values and severity of liver diseases has been also reported in recent two studies[[17](#_ENREF_17),[18](#_ENREF_18)].

In this study we aimed to investigate the clinical utility of RDW, an inexpensive and easily available marker, in indicating the presence of NASH by comparing with simple steatosis and healthy controls. And we also proposed to evaluate whether there isan association between RDW values and fibrotic stages and histological features of NASH.

**MATERIALS AND METHODS**

***Patients***

This retrospective study was performed in Gazi University Department of Gastroenterology a tertiary reference center (Ankara, Turkey) between January 2010 and May 2013. All the patients who had persistently elevated liver enzymes and hepatosteatosis on ultrasonography in the absence of any causes of elevated aminotransferases were evaluated in the study. Among them, patients who had histopathologically consistent with NASH and simple steatosis included in the study. Control group was created among individuals who had normal aminotransferase levels and normal abdominal ultrasonography. Patients who diagnosed with viral hepatitis, sclerosing cholangitis, primary biliary cirrhosis, autoimmune hepatitis, hemochromatosis, Wilson’s disease, alpha-1-antitrypsin deficiency, malignancy and drug-induced liver disease were excluded. Alcohol consumption of more than > 20 g/d for men, and > 10 g/d for women, presence of infectious diseases on admission, chronic renal diseases, collagen vascular diseases, malignancies, hematological diseases those might impair red cell production (such as iron deficiency, B12 or folat deficiency), increased red cell destruction, hemoglobinopathies, blood transfusions, bone marrow depression, usage of anticoagulant drugs, non-steroid anti-inflammatory drugs and hepatotoxic drugs were also considered as exclusion criteria. Patients who had diabetes mellitus were on oral antidiabetics and/or insulin therapy. Patients who had hypertension were all using ACE inhibitors and under control in terms of hypertension.

***Clinical and laboratory assessment***

Demographical, clinical and laboratory data were all collected and registered in a database by an uninformed clinician to prevent the bias. Body mass index was calculated by dividing weight in kilograms (kg) by square of length in meter (m) as (kg/m2).

Before the liver biopsy venous blood samples were obtained from antecubital vein from all patients between 8.30 and 10.00 am, after fasting for at least 8-12 h. A CBC containing; RDW, hemoglobin (HB), white blood cell (WBC), neutrophils, lymphocytes, platelets and mean platelet volume (MPV) was performed with Beckman Coulter (High Wycombe, United Kingdom) Gen-S automated analyzer within 2 hours after obtaining blood samples. RDW was calculated by Beckman Coulter automated analyzer as an equation of RDW = SD/MCV × 100.

Albumin, creatinine, blood urine nitrogen (BUN), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl aminotransferase (GGT) and alkaline phosphatase (ALP) were determined by a hospital auto analyzer of Roche Modular System. All the laboratory analysis were performed in our hospital’s hematology laboratory.

***Histopathologic evaluation of liver***

Percutaneous liver biopsy was performed using a 16-G disposable needle by a skilled clinician. All specimens were 20- 25 mm in length and contained at least of 11 portal tracts. All liver tissue samples were evaluated by the same experienced hepatopathologist whom was uninformed to patients’ clinical and also laboratory data. Formalin-fixed paraffin-embedded liver tissues were histologically diagnosed by Hematoxylin&Eosin (HE) and Masson trichrome stains, and the diagnosis of NASH was evaluated on Brunt’s Criteria[[19](#_ENREF_19)]. Histological characteristics were graded according to the NAFLD scoring system recommended by the National Institute of Diabetes and Digestive and Kidney Diseases NASH Clinical Research Network[[20](#_ENREF_20)]. Steatosis was graded as 0 = < 5%; 1=>33%-66%; 3 = >66% lobular inflammation was graded as 0 = no foci; 1 = <2 foci; 2 = 2-4 foci; 3=>4foci; andballooning was graded as 0=none; 1=few ballooning cells; 2 = many ballooning cells respectively.

Depending on the recommendations of Brunt’s criteria steatosis (0–3), lobular inflammation (0–3) and ballooning (0–2) were then combined to establish the NAFLD activity score (0–8). Fibrosis was also scored as 0 = no fibrosis; 1 = periportal or perisinusoidal fibrosis; 2 = perisinusoidal and portal/periportal fibrosis; 3 = bridging fibrosis; and 4 = cirrhosis**.** While mild fibrosis was defined with fibrotic score ≤ 1 advanced fibrosis was defined with fibrotic score > 1. Mild fibrosis due to NASH is shown in Figure 1A and advanced fibrosis in Figure 1B.

***Ethics***

The study was in accordance withtheethical guidelines of the 1975 Declaration of Helsinki and approved by the institutional review board.

***Statistical analysis***

Statistical analysis were performed using the SPSS software version 17 and MedCalc version 12. The variables were investigated by using visual (histograms, probability plots) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk’s test) to determine whether or not they were normally distributed. Non-normally distributed and non-parametric variables were compared between groups by using Mann-Whitney U test where appropriate. The *χ2* test where appropriate was used to compare the propositions in different groups. As the RDW was not normally distributed the Kruskal – Wallis test was conducted to compare among NASH, simple steatosis and healthy control groups. The Mann-Whitney U test was used to test the significance of pairwise differences using Bonferroni correction to adjust for multiple comparisons. For the multivariate analysis, the possible factors identified with univariate analyses were further entered into the logistic regression analysis to determine independent predictors of NASH. To assess model fit we used Hosmer-Lemeshow goodness of fit statistics. Spearman rank correlation coefficients (*r*)were calculated to assess the correlation between RDW and liver histopathological features (inflammation, NAS score, thedegree of steatosis and degree of fibrosis) and clinical characteristics of NASH patients. Receiver operating characteristics (ROC) analysis was used to evaluate the role of RDW in distinguishing subjects with NASH and fibrosis. And also ROC analysis was used in distinguishing advanced and mild fibrotic stage groups. Cutoff values that maximized both sensitivity and specificity were chosen NASH group. A *P* < 0.05 was considered statistically significant.

**RESULTS**

***Patient characteristics and liver histology***

Sixty-two biopsy proven NASH patients, 32 biopsy proven simple steatosis patients and 30 healthy controls were recruited in the present study. The median ages of patients with NASH, simple steatosis and healthy controls were 49.5 (22-77), 48 (24-71) and 48 (24-72), respectively. There was no statistically significant difference between the ages of participants of the study. The mean BMI (kg/m2) of NASH, simple steatosis and healthy controls were 27.13 ± 0.37, 25.97 ± 0.67 and 24.22 ± 0.45, respectively (*P* < 0.01). The platelet counts among NASH, simple steatosis and healthy controls were 245.5 ± 34.73, 244.5 ± 13.8 and 260 ± 4.47, respectively (*P* > 0.05). But there was an inversely significant correlation between platelet counts and fibrotic scores (*r* = -0.335 and *P* <0.01). Among the NASH group 30/62 (48.3%), steatosis group 18/32 (56.2%) and healthy controls 18 (60 %) were men (*P* > 0.05) and the median RDW values were; 14.28 ± 0.25, 13.37 ± 0.12 and 12.96 ± 0.14, respectively *P* < 0.01. Clinical and laboratory data of patients with NASH, simple steatosis and healthy control groups are summarized in Table 1. The comparison of RDW values among NASH, simple steatosis and healthy control groups is also shown in Figure 2A.

***Relationship between RDW and the presence of NASH and fibrosis***

The ROC analysis suggested that a cutoff value of 13.56 has the highest sensitivity (61.3%) and specificity (72.6%) for detecting patients with NASH with an area under the curve (AUC) of 0.70 (95% Confidence Intervals (CI): 0.600 – 0.805), (*P* < 0. 01) as shown in Figure 3A.

According to the liver histopathological features while mild fibrosis group was consisted of 40 patients there were 22 patients in advanced fibrosis group. We compared RDW values between mild and advanced fibrosis subgroups of NASH and we found statistically significant difference 13.95 ± 1.74 and 15.85 ± 1.89, respectively (*P* < 0.01) as seen in Figure 2B. The ROC curve of RDW for the identification of advanced fibrosis in NASH was statistically significant; AUC = 0.78, 95 %CI: 0.660 -0.903, *P* < 0.01 as seen in Figure 3B.

***Correlation between RDW and clinical/histopathological features of patients with NASH***

In Spearman correlation analysis, there were statistically significant correlations between RDW values and fibrotic scores, age and gender as seen in Table 2. The correlation between fibrotic scores and RDW values reached statistically significance such that Spearman’s correlation coefficients were *r* = 0.42, and *P*< 0.001 as seen in Figure 4.

According to the univariate analysis for the presence of NASH; age, gender, platelet counts, ALT, AST, ALP, GGT, albumin, BMI and RDW were statistically significant predictor factors of the presence of the NASH. All the results of univariate analysis are shown in Table 3.

The variables those were statistically significant in univariate logistic regression analyses furthered entered into the multivariate logistic regression analysis and RDW continued to be statistically significant and an independent predictor of fibrosis (OR = 1.75, 95%CI:1.129–2.711, *P* < 0.001) as seen in Table 4.

**DISCUSSION**

In the present study, we have concluded that patients with biopsy proven NASH have higher RDW values when compared with simple steatosis and healthy control groups. Also the increase in RDW values is correlated with disease progression as the patients with advanced fibrosis have higher values of RDW than mild fibrosis group. There is a positive correlation between RDW values and fibrotic scores. As a result of multivariate logistic regression analysis RDW is an independent predictor of NASH and advanced liver fibrosis.

The search for a noninvasive diagnostic marker indicating the histological changes and fibrotic stages observed in NASH is very important. Because of the absence of approved treatment methods for NASH, care must be taken on the detection of advanced fibrosis. The risk stratification requires an adequate evaluation of fibrosis which is at present just possible by performing liver biopsy. Because of being an invasive method with natural risks, costs, and disturbing for the patients, a great attention should be taken for the development of noninvasive alternatives of determining the degree of liver fibrosis.

Lou *et al*[[21](#_ENREF_21)] have found that RDW values were significantly increased in patients with hepatitis B and also high RDW values were associated with disease severity. Similarly Chen *et al*[[22](#_ENREF_22)] have concluded that RDW to platelet index a routinely available and easily calculated index can predict significant liver fibrosis due to chronic hepatitis B with a high accuracy. They also have estimated that this index may reduce the requirement of liver biopsy in patients infected with chronic hepatitis B. High RDW values are associated with poor outcomes in patients with cardiovascular diseases[[23](#_ENREF_23), [24](#_ENREF_24)]. Although the main mechanism that cause elevation of RDW levels in these different conditions is unknown, it is speculated that inflammation may play an important role in this course[[25](#_ENREF_25)]. There are some pathways to be accused to explain the increase in RDW values such as; impairing iron metabolism, suppressing erythropoietin gene expression, inhibiting proliferation of erytroid progenitor cells, down-regulating erythropoietin receptor expression and reducing erythrocyte circulatory half-life[[26](#_ENREF_26)].

Alkhouri *et al*[[27](#_ENREF_27)] found that neutrophil to lymphocyte ratio was higher in patients with NASH and advanced fibrosis. They hypothesized that this ratio could be used as a novel noninvasive marker to predict advanced liver fibrosis due to NASH. Tonelli *et al*[[28](#_ENREF_28)] reported that higher RDW values might reflect an underlying chronic inflammation, which could result in an increased risk of cardiovascular disease.

These results suggest that inflammation may be a potential underlying biological mechanism for increased RDW values. Chronic inflammation may play a role in disease progression of NASH and fibrosis. Actually, multiple inflammatory cytokines are assessed in different studies as noninvasive markers for the presence of NASH and fibrosis[[29](#_ENREF_29)]. Herewith, increased RDW values may be considered as a chronic inflammatory process in the pathogenic basis of NASH. Moreover our findings revealed that RDW seems to be related in discriminating fibrotic scores of NASH, it could be a useful marker of predicting the progression and fibrotic stages of NASH.

We hypothesized that the relationship between RDW and NASH may be a result of an effect of inflammatory process by suppressing mature erythrocytes, secreting young erythrocytes into circulation and so leading to anisocytosis and high RDW values. To the best of our knowledge this is the first study evaluating an association between high RDW values and high fibrotic scores in a well designed specifically organized patient group of biopsy proven NASH.

There are some limitations of our study that RDW was assessed on a single situation instead of multiple consecutive measurements, therefore we couldn’t evaluate any biologic variabilities and measurement errors which could be seen as a result of this. Also erythropoietin, reticulocyte count, inﬂammatory markers such as IL-1, IL-6, TNF-α were not provided, they might give us some important information about the pathophysiology of underlying high RDW physiology. A lack of markedly fall in the platelet count of NASH patients may be due to the small sample size and small number of cirrhotic patients. This must be evaluated in large population based prospective studies.

In summary, our study showed that in patients with biopsy proven NASH, RDW is associated with histological severity and can be used to identify patients with advanced liver fibrosis. Unlike many other noninvasive markers of NAFLD, the RDW is inexpensive, widely available and easily repeatable. Although the accuracy of RDW for detecting NASH and significant fibrosis is not enough, RDW in combination with other markers may help to identify the patients at increased risk of having advanced disease. Maybe independent of anemia and other factors RDW could be used in estimating the fibrotic process of different diseases, it may become one of the corn stores of inflammation and fibrosis by confirmation of large population based studies in the future.

**COMMENTS**

***Background***

Non-alcoholic steatohepatitis (NASH) became one of the most common cause of chronic liver diseases. Liver biopsy is the only method for distinguishing NASH from other diagnoses and evaluating the damage and fibrosis of liver but it may have procedure related clinical complications.

***Research frontiers***

Therefore, it has been attempted to find noninvasive methods for detecting the presence of NASH, liver fibrosis and avoiding procedure related complications. In this study, we demonstrated that red cell distribution width (RDW) has indicating property in the diagnosis of NASH and advanced liver fibrosis.

***Innovations and breakthroughs***

Recent reports have demonstrated that elevated RDW values were related to negative outcomes in cardiovascular and metabolic disorders, colon cancer and stroke independent of hemoglobin values. The association between the RDW values and severity of liver diseases has been also reported in recent two studies. This is the first study demonstrating the importance of RDW in the diagnosis of NASH and predicting the advanced liver fibrosis.

***Applications***

By understanding that high RDW values can be seen in the presence of NASH and advenced liver fibrosis, this study may represent a future strategy for non-invasive diagnostic method of patients with NASH.

***Terminology***

RDW is a widely available, inexpensive and easily repeatable parameter of variation of circulating red cells demonstrating the heterogeneity of red cell volume a component of the complete blood count. Chronic inflammation plays a significant role in disease progression to NASH. Liver fibrosis is a result of chronic injury and only can be diagnosed invasively by liver biopsy.

***Peer review***

The results presented a new laboratorial and non-invasive marker of NASH and fibrotic stage. The authors concluded that in patients with biopsy proven NASH, RDW is associated with histological severity and can be used to identify patients with advanced liver fibrosis disease.This result is interesting and RDW may be used as a non-invasive marker of indicating the presence of NASH and advanced liver fibrosis.

**REFERENCES**

1 **Brunt EM**. Nonalcoholic steatohepatitis: pathologic features and differential diagnosis. *Semin Diagn Pathol* 2005; **22**: 330-338 [PMID: 16939061]

2 **Browning JD**, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, Grundy SM, Hobbs HH. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 2004; **40**: 1387-1395 [PMID: 15565570 DOI: 10.1002/hep.20466]

3 **Clark JM**. The epidemiology of nonalcoholic fatty liver disease in adults. *J Clin Gastroenterol* 2006; **40 Suppl 1**: S5-10 [PMID: 16540768]

4 **Lazo M**, Clark JM. The epidemiology of nonalcoholic fatty liver disease: a global perspective. *Semin Liver Dis* 2008; **28**: 339-350 [PMID: 18956290 DOI: 10.1055/s-0028-1091978]

5 **Zhou YJ**, Li YY, Nie YQ, Ma JX, Lu LG, Shi SL, Chen MH, Hu PJ. Prevalence of fatty liver disease and its risk factors in the population of South China. *World J Gastroenterol* 2007; **13**: 6419-6424 [PMID: 18081233]

6 **Zafrani ES**. Non-alcoholic fatty liver disease: an emerging pathological spectrum. *Virchows Arch* 2004; **444**: 3-12 [PMID: 14685853 DOI: 10.1007/s00428-003-0943-7]

7 **McCullough AJ**. Pathophysiology of nonalcoholic steatohepatitis. *J Clin Gastroenterol* 2006; **40 Suppl 1**: S17-S29 [PMID: 16540762]

8 **Ekstedt M**, Franzén LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, Kechagias S. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* 2006; **44**: 865-873 [PMID: 17006923 DOI: 10.1002/hep.21327]

9 **Day CP**. Pathogenesis of steatohepatitis. *Best Pract Res Clin Gastroenterol* 2002; **16**: 663-678 [PMID: 12406438]

10 **Feldstein AE**, Lopez R, Tamimi TA, Yerian L, Chung YM, Berk M, Zhang R, McIntyre TM, Hazen SL. Mass spectrometric profiling of oxidized lipid products in human nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *J Lipid Res* 2010; **51**: 3046-3054 [PMID: 20631297 DOI: 10.1194/jlr.M007096]

11 **Wieckowska A**, Papouchado BG, Li Z, Lopez R, Zein NN, Feldstein AE. Increased hepatic and circulating interleukin-6 levels in human nonalcoholic steatohepatitis. *Am J Gastroenterol* 2008; **103**: 1372-1379 [PMID: 18510618 DOI: 10.1111/j.1572-0241.2007.01774.x]

12 **Felker GM**, Allen LA, Pocock SJ, Shaw LK, McMurray JJ, Pfeffer MA, Swedberg K, Wang D, Yusuf S, Michelson EL, Granger CB. Red cell distribution width as a novel prognostic marker in heart failure: data from the CHARM Program and the Duke Databank. *J Am Coll Cardiol* 2007; **50**: 40-47 [PMID: 17601544 DOI: 10.1016/j.jacc.2007.02.067]

13 **Ani C**, Ovbiagele B. Elevated red blood cell distribution width predicts mortality in persons with known stroke. *J Neurol Sci* 2009; **277**: 103-108 [PMID: 19028393 DOI: 10.1016/j.jns.2008.10.024]

14 **Spell DW**, Jones DV, Harper WF, David Bessman J. The value of a complete blood count in predicting cancer of the colon. *Cancer Detect Prev* 2004; **28**: 37-42 [PMID: 15041076 DOI: 10.1016/j.cdp.2003.10.002]

15 **Lee WS**, Kim TY. Relation between red blood cell distribution width and inflammatory biomarkers in rheumatoid arthritis. *Arch Pathol Lab Med* 2010; **134**: 505-506 [PMID: 20367302]

16 **Malandrino N**, Wu WC, Taveira TH, Whitlatch HB, Smith RJ. Association between red blood cell distribution width and macrovascular and microvascular complications in diabetes. *Diabetologia* 2012; **55**: 226-235 [PMID: 22002006 DOI: 10.1007/s00125-011-2331-1]

17 **Hu Z**, Sun Y, Wang Q, Han Z, Huang Y, Liu X, Ding C, Hu C, Qin Q, Deng A. Red blood cell distribution width is a potential prognostic index for liver disease. *Clin Chem Lab Med* 2013; **51**: 1403-1408 [PMID: 23314558 DOI: 10.1515/cclm-2012-0704]

18 **Milić S**, Mikolasević I, Radić M, Hauser G, Stimac D. Clinical utility of red cell distribution width in alcoholic and non-alcoholic liver cirrhosis. *Coll Antropol* 2011; **35 Suppl 2**: 335-338 [PMID: 22220466]

19 **Brunt EM**, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999; **94**: 2467-2474 [PMID: 10484010 DOI: 10.1111/j.1572-0241.1999.01377.x]

20 **Kleiner DE**, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313-1321 [PMID: 15915461 DOI: 10.1002/hep.20701]

21 **Lou Y**, Wang M, Mao W. Clinical usefulness of measuring red blood cell distribution width in patients with hepatitis B. *PLoS One* 2012; **7**: e37644 [PMID: 22649548 DOI: 10.1371/journal.pone.0037644]

22 **Chen B**, Ye B, Zhang J, Ying L, Chen Y. RDW to Platelet Ratio: A Novel Noninvasive Index for Predicting Hepatic Fibrosis and Cirrhosis in Chronic Hepatitis B. *PLoS One* 2013; **8**: e68780 [PMID: 23874760 DOI: 10.1371/journal.pone.0068780]

23 **Hampole CV**, Mehrotra AK, Thenappan T, Gomberg-Maitland M, Shah SJ. Usefulness of red cell distribution width as a prognostic marker in pulmonary hypertension. *Am J Cardiol* 2009; **104**: 868-872 [PMID: 19733726 DOI: 10.1016/j.amjcard.2009.05.016]

24 **Cheng CK**, Chan J, Cembrowski GS, van Assendelft OW. Complete blood count reference interval diagrams derived from NHANES III: stratification by age, sex, and race. *Lab Hematol* 2004; **10**: 42-53 [PMID: 15070217]

25 **Lippi G**, Targher G, Montagnana M, Salvagno GL, Zoppini G, Guidi GC. Relation between red blood cell distribution width and inflammatory biomarkers in a large cohort of unselected outpatients. *Arch Pathol Lab Med* 2009; **133**: 628-632 [PMID: 19391664 DOI: 10.1043/1543-2165-133.4.628]

26 **Weiss G**, Goodnough LT. Anemia of chronic disease. *N Engl J Med* 2005; **352**: 1011-1023 [PMID: 15758012]

27 **Alkhouri N**, Morris-Stiff G, Campbell C, Lopez R, Tamimi TA, Yerian L, Zein NN, Feldstein AE. Neutrophil to lymphocyte ratio: a new marker for predicting steatohepatitis and fibrosis in patients with nonalcoholic fatty liver disease. *Liver Int* 2012; **32**: 297-302 [PMID: 22097893]

28 **Tonelli M**, Sacks F, Arnold M, Moye L, Davis B, Pfeffer M. Relation Between Red Blood Cell Distribution Width and Cardiovascular Event Rate in People With Coronary Disease. *Circulation* 2008; **117**: 163-168 [PMID: 18172029 DOI: 10.1161/CIRCULATIONAHA.107.727545]

29 **Wieckowska A**, McCullough AJ, Feldstein AE. Noninvasive diagnosis and monitoring of nonalcoholic steatohepatitis: present and future. *Hepatology* 2007; **46**: 582-589 [PMID: 17661414 DOI: 10.1002/hep.21768]

**P-Reviewers** Castiella A, Ji G, Nakano S

**S-Editor** Zhai HH **L-Editor E-Edito**r

**Figure 1 Mild perivenular-perisinusoidal fibrosis (A) and advanced perivenular-perisinusoidal, and periportal fibrosis (B) in non-alcoholic steatohepatitis. A: (trichrome stain, x400).**

**Figure 2 Red cell distribution width values of healthy controls, simple steatosis and NASH groups (A), and fibrotic subgroups of non-alcoholic steatohepatitis (B).**

**Figure 3 Receiver operating characteristics curve of Red cell distribution width values for the identification of patients with non-alcoholic steatohepatitis (A), and the identification of fibrosis in non-alcoholic steatohepatitis(B).**

**Figure 4 Correlation between red cell distribution width values and fibrotic scores of non-alcoholic steatohepatitis.**

**Table1 Demographic and laboratory features of non-alcoholic steatohepatitis, simple steatosis and healthy control groups**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Factor** | **NASH group (*n* = 62)** | **Steatosis group** **(*n* = 32)** | **Healthy (*n*=30) control group** |  ***P-*value** |
| Age mean ± SD | 48.81 ± 12.21  | 47.25 ± 12.58  | 48.03 ± 9.54 | NS |
| Gender (male) | 30 (48.3) |  18 (56.2) | 18 (60 ) | NS |
| BMI (kg/m2) | 27.13 ± 0.37 | 25.97 ± 0.67  | 24.22± 0.45 | < 0.01 |
| Hemoglobin (g/dL) | 14.10 ± 0.16  | 14.51 ± 0.28 | 13.91 ± 0.11 | NS |
| Platelet (x103/mL) | 245.5 ± 34.73 | 244.5 ± 13.8 | 260 ± 4.47 | NS |
| WBC (k/UL)  | 6950± 319 | 7250 ± 330 | 7350 ± 146 | NS |
| Neutrophil ratio  | 59.39 ± 1.20 | 59.87 ± 1.20 | 59 ± 1.1 | NS |
| Lymphocyte ratio  | 31.29 ± 1.09 | 29.88 ± 1.10  | 32 ± 0.78 | NS |
| RDW (%) | 14.28 ± 0.25 | 13.37 ± 0.12  | 12.96 ± 0.14 | < 0.01 |
| MPV (fl) | 8.63 ± 1.30  | 8.49 ±1.06  | 8.20 ± 0.59 | NS |
| MCV (fl)  | 86.34 ± 0.88 | 87.98 ± 1.37 | 85.5 0.82 | NS |
| ALT (IU/L)  | 54 ± 8.1 | 45 ± 3.97 | 32.5 ± 1.49 | <0.01 |
| AST (IU/L)  | 40.05 ± 6.04  | 40.05 ± 3.01  | 24 ± 1.39 | < 0.01 |
| ALP (IU/L)  | 87.5± 9.09 | 81.5 ± 3.91 | 61 ±1.87 | <0.01 |
| GGT (IU/L)  | 61 ± 13.4 | 32 ± 3.4 | 27 ± 2.5 | <0.01 |
| Albumin (g/dL) | 4.36 ± 0.73 | 4.5 ± 0.42  | 4.9 ± 0.75 | <0.01 |
| Creatinine  | 0.78 **±** 0.033 | 0.79 **±** 0.023 | 0.72 **±** 0.014 | NS |
| BUN (mg /dL)  | 12.25 ± 0.85 | 14.2 ± 0.67 | 13.46 ± 0.71 | NS |
| Diabetes mellitus | 19 (30.6 ) | 9 (28.1 ) | 0 (0 ) | NS |
| Hypertension | 22 (35.4 ) | 7 (21.8 ) | 0 (0 ) | NS |

Values are presented as *n* (%) frequencies, median ± SE for skewed distributed variables. BMI: Body-mass index; RDW: red cell distribution width; MPV: Mean platelet volume; NS: Not significant.

**Table 2 Red cell distribution width in correlation with clinical characteristics and histological features of non-alcoholic steatohepatitis**

|  |  |  |
| --- | --- | --- |
| Factor | *r* -value | *P*-value |
| Age (yr) | 0.245 | < 0.01 |
| BMI (kg/m2) | 0.131 | NS |
| Gender  | -0.251 | < 0.01 |
| Fibrosis | 0.579 | < 0.01 |
| Inflammation | 0.207 | NS |
| Steatosis | 0.121 | NS |
| Ballooning | 0.175 | NS |
| NASH | 0.217 | NS |

BMI: Body mass index; RDW: Red cell distribution width; NASH: non-alcoholic steatohepatitis; NS: Not significant.

**Table 3 Univariate analyses results of predicting liver fibrosis**

|  |  |  |  |
| --- | --- | --- | --- |
|  | OR  |  95%CI | *P* - value |
| Age | 1.05 | 1.004-1.109 | < 0.05 |
| Gender | 1.46 | 0.516-4.171 |  NS |
| Platelet | 0.98 | 0.988-0.996 | < 0.01 |
| ALT | 1.08 | 0.976-1.004 |  NS |
| AST | 0.98 | 0.963-1.007 |  NS |
| ALP | 1.015 | 1.002-1.028 | < 0.05 |
| GGT | 1.002 | 0.998-1.007 |  NS |
| RDW | 1.73 | 1.242-2.409 | < 0.01 |
| Albumin | 0.418 | 0.158-1.105 |  NS |
| BMI | 1.012 | 0.847-1.209 |  NS |

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; GGT: gamma glutamyl aminotransferase; RDW: Red cell distribution width; BMI: Body mass index; NS: Not significant.

**Table 4 Multivariate analyses results depending on advanced liver fibrosis**

|  |  |  |  |
| --- | --- | --- | --- |
|  | OR |  95%CI  | *P*-value |
| ALP | 1.005 | 0.993-1.018 | NS |
| RDW | 1.75 | 1.129-2.711 | <0.05 |
| Age | 1.020 | 0.957-1.086 | NS |
| Platelet | 0.991 | 0.981-1.001 | NS |
| Albumin | 1.016 | 0.291-3.545 | NS |

ALP: Alkaline phosphatase; RDW: Red cell distribution width; NS: Not significant.