Abstract. – OBJECTIVE: Red cell distribution width (RDW), an index of erythrocyte size, is recently found to be associated with inflammation and a high risk for cardiovascular disease. Hyperglycemia, the hallmark of prediabetes (PDM) and diabetes mellitus (DM), causes endothelial dysfunction and a proinflammatory state. We investigated the relationship between RDW and hs-CRP in patients with prediabetes and overt DM. PATIENTS AND METHODS: A total of 155 patients were categorized into 3 groups according to the 2007 guideline for American Diabetes Association: “Type 2 DM” group (n = 45), “PDM” group (n = 60) and “Control” group (n = 50). RDW and hs-CRP levels were measured. RESULTS: PDM group had higher hs-CRP and RDW levels than the control group (14.3 ± 0.84 vs. 12.7 ± 0.8, p < 0.001 for RDW; 0.91 ± 0.49 vs. 0.55 ± 0.37, p < 0.001 for hs-CRP). Similarly, when compared with the PDM, RDW and hs-CRP levels were higher in the DM group (14.8 ± 0.87 vs. 14.3 ± 0.84, p = 0.002 for RDW; 1.15 ± 0.59 vs. 0.91 ± 0.49, p = 0.03 for hs-CRP). CONCLUSIONS: Prediabetes and diabetes were associated with elevated RDW levels which may be attributed to a subclinical inflammatory background.

Key Words:
Diabetes mellitus, hs-CRP, Inflammation, Prediabetes, RDW.

Introduction

Diabetes mellitus (DM) is a significant health concern that is associated with increased mortality and morbidity for cardiovascular diseases, stroke, kidney failure, and blindness1. The best coping strategy with diabetes is early diagnosis and prevention rather than dealing with complications2. Since the progressive nature of impairment in insulin secretion and loss of beta-cells, only about 50% percent of beta-cell function remains at the time of diagnosis in type 2 DM1. Therefore, prediabetes, a clinical condition with higher glucose levels than usual, is of great clinical importance in early diagnosis and prevention of DM. There is solid evidence that glycemic control in prediabetes can prevent or delay the development of overt DM and its complications3,4,5.

Red cell distribution width (RDW) is a routine parameter of complete blood count analysis (CBC) which reflects the variability of circulating red blood cell sizes. Traditionally RDW is used in the differential diagnosis of anemia6. A high RDW value can be seen not only in hematological disorders (Iron, vitamin B12 or folate deficiency, impaired red blood cell production due to hemolysis), but also in some clinical conditions such as prehypertension, hypertension, thrombotic thrombocytopenic purpura (TTP), and pregnancy7-10. Recently, it has been reported that higher RDW values mean poor outcomes in acute myocardial infarction, coronary artery disease, and heart failure11-14.

In our study, our aim is to research the relationship between RDW and hs-CRP in patients with prediabetes and overt DM.

Patients and Methods

A total of 155 patients of outpatient clinics were included in this prospective randomized controlled study. The subjects were categorized into three groups: 45 patients with Type 2 DM, 60 patients with prediabetes and 50 age-, and sex-matched subjects without prediabetes or DM. Prediabetic and diabetic patients were determined according to the 2007 guidelines in American Diabetes Association15. Those criteria are as follows.

Prediabetes
(1) Impaired fasting glucose:
Fasting plasma glucose 100-125 mg/dL (5.6-6.9 mmol/L)
(Fasting = no caloric intake for ≥ 8 hours)
(2) Impaired glucose tolerance:
   2-hour plasma glucose 140-199 mg/dL
   (7.8-11.0 mmol/L)
(Measured by oral glucose tolerance test using a 75-g glucose load)

**Diabetes**
(1) Fasting plasma glucose:
   ≥126 mg/dL (≥ 7.0 mmol/L)

(2) 2-hour plasma glucose:
   ≥200 mg/dL (≥ 11.0 mmol/L)

The subjects were included in the prediabetes group in the presence of impaired fasting glucose, impaired oral glucose tolerance test (OGTT) or both.

**Exclusion Criteria**
The exclusion criteria were: prehypertension or HT (according to JNC-7 guideline), coronary artery disease, manifest heart disease, hepatic failure, TTP, chronic obstructive lung disease, renal failure, pregnancy, hyperthyroidism, anemia, any prior blood transfusion, any hypertensive medication.

All participants included in the study were informed about the study and their verbal and written consent was obtained to participate in the study voluntarily. This study was approved by the Research Ethics Review Board of Mustafa Kemal University (Approval #2016-1/3).

After questioning the clinical history of risk factors such as age, gender, hypercholesterolemia, smoking and family history, previous medications, height and weight were measured for each participant. BMI was calculated by dividing weight in Kilograms by size in square meters (kg/m²). The glomerular filtration rate (GFR) was estimated by the simplified Modification of Diet in Renal Disease Equation. White blood cell (WBC), Hemoglobin (Hb), and RDW count were evaluated as part of the automated complete blood count analyzed by the Beckman-Coulter Gen-S system device (Beckman-Coulter Inc., California, CA, USA). Anemia was defined as a baseline Hb < 12 g/dL in women and Hb < 13 g/dl in men by the criteria of the World Health Organization (WHO).

Serum total cholesterol, TG, LDL, HDL, glucose, urea, creatinine, levels and hs-CRP were evaluated from venous blood samples taken in the morning after eight hours of fasting. Serum creatinine, Blood urea nitrogen, levels of high-density lipoprotein, triglycerides, low-density lipoprotein, and thyroid-stimulating hormone (TSH) were recorded. hs-CRP was evaluated in serum by EIA (Image hs-CRP EIA kit, Beckman Coulter Inc., California, CA, USA). Transthoracic echocardiography was performed before coronary angiography and biplane Simpson ejection fraction (%) was calculated. Our study was approved by the local ethics committee.

**Statistical Analysis**
Statistical analysis was conducted with SPSS 13 (SPSS Inc, Chicago, IL, USA) software package program. Whereas categorical variables were presented as percentages, continuous variables were expressed as mean ± standard deviation. Chi-square (χ²) test was employed for the comparison of categorical variables. Differences in numerical variables were evaluated with the Mann-Whitney U test or Kruskal-Wallis analysis of variance, as appropriate. To determine the independent predictors of prediabetes, uni- and multivariate analyses were performed. Parameters found to be significant (p<0.10) in univariate analysis were evaluated with stepwise logistic regression analysis. Ninety-five percent confidence intervals and odds ratios (OR) are presented. An exploratory assessment of additional breakpoints was performed using receiver operating characteristics (ROC) curve analysis. A p < 0.05 was considered statistically significant.

**Results**
In total, 155 patients (42.4 ± 3.0 years, 62.6% comprised of males) were included in the study. Baseline clinical and laboratory characteristics relative to groups were shown in Table I. Age, sex, smoking status, HDL-C, LDL-C, triglyceride, hemoglobin, GFR, and TSH levels were not different between the groups. Glucose levels, HbA1c, and BMI were higher in the prediabetic group. The patients with DM had serum hs-CRP and higher RDW levels than the prediabetic patients (14.8 ± 0.87 vs. 14.3 ± 0.84, p = 0.002 for RDW; 1.15 ± 0.59 vs. 0.91 ± 0.49, p = 0.03 for hs-CRP) and control subjects (14.8 ± 0.87 vs. 12.7 ± 0.8, p < 0.001 for RDW; 1.15 ± 0.59 vs. 0.55 ± 0.37, p < 0.001 for hs-CRP). In addition, the RDW levels were higher in the prediabetic group than the control group (14.3 ± 0.84 vs. 12.7 ± 0.8, p < 0.001) (Figure 1). Similarly, serum hs-CRP levels were higher in prediabetic group than the control group, as well (0.91 ± 0.49 vs. 0.55 ± 0.37, p < 0.001) (Figure 1). Uni-
variate correlation analysis revealed a positive correlation between RDW levels and hs-CRP levels ($\rho=0.41$, $p < 0.001$).

The ROC curve analysis further revealed that RDW was a strong indicator of the “prediabetic or diabetic state” in patients with an AUC of 0.92 (95% CI: 0.88 to 0.97) (Figure 2). The optimal threshold of RDW level that maximized the combined specificity and sensitivity to predict the “prediabetic or diabetic state” was 13.5%. Sensitivity, specificity, positive predictive value, and negative predictive value to identify “prediabetic or diabetic” patients were 88%, 85%, 93%, and 76%, respectively.

For determining the predictors of “prediabetic or diabetic state,” uni- and multivariate analysis were performed. For predicting the “prediabetic or diabetic state”, the RDW score was dichotomized into high (RDW $\geq 13.5$) and low (RDW < 13.5) groups. Parameters showing significance in multivariate analysis, univariate analysis (age, BMI, hs-CRP and TSH) were evaluated by multivariate analysis to determine the independent predictors of “prediabetic or diabetic state”. Thus, hs-CRP, BMI, and TSH were found to be independent predictors of “prediabetic or diabetic state” (Table II).

**Discussion**

In this study, we investigated the relationship between RDW and hs-CRP in prediabetic and diabetic patients. This study showed that prediabetics and diabetes were associated with elevated RDW levels which may be attributed to a subclinical inflammatory background.

DM is a chronic illness caused by defects in insulin secretion and loss of beta-cells, resulting in high glucose levels\(^3\). It is a significant health concern because of the associated high mortality and morbidity for cardiovascular diseases, stroke, kidney failure, and blindness\(^1\). Due to the chronic, insidious and progressive course of the disease, many organ systems are affected and irreversibly damaged in the presence of poor glycemic control. Therefore, early diagnosis is crucial for preventing life-threat-
enening complications because only about 50% percent of beta-cell function remains at the time of diagnosis in type 2 DM. Although the exact molecular basis of the end-organ damage is not fully understood, for the time being, the primary target of hyperglycemia appears to be the endothelial cell in which it causes fundamental structural changes, resulting in endothelial dysfunction and microangiopathy. This condition, in turn, causes a prothrombotic and proinflammatory state that predisposes to prediabetes. Because the hallmark of prediabetes and DM is the hyperglycemic state. As mentioned above, hyperglycemia leads to endothelial dysfunction and proinflammatory state, even though overt type 2 DM precedes "prediabetes", an inflammatory background is present at the very beginning of the disease. In the previous studies, hs-CRP, a well-known inflammatory marker, was associated with prediabetes and elevated blood glucose levels, showing the inflammatory background. Following the literature, in our study, we found that hs-CRP levels were higher in both prediabetic and diabetic patients when compared to normoglycemic subjects. Moreover, diabetic patients had higher hs-CRP levels than the prediabetic subjects. These findings support the idea that inflammation, as a reason or consequence, coexists in all the progression of the disease, and as the disease proceed, more inflammation coexists.

Similarly, RDW values were higher in prediabetic and diabetic patients than in the controls. RDW is an index for the size variability of circulating erythrocytes and is routinely reported by CBC analysis. Although commonly used in the differential diagnosis of anemias,

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate OR (95% CI)</th>
<th>p</th>
<th>Stepwise multivariate OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prediabetic and diabetic state</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.13 (1.0-1.28)</td>
<td>0.04</td>
<td>1.19 (1.1-1.3)</td>
<td>0.03</td>
</tr>
<tr>
<td>BMI</td>
<td>1.13 (1.04-1.24)</td>
<td>0.003</td>
<td>0.69 (0.48-0.98)</td>
<td>0.04</td>
</tr>
<tr>
<td>TSH</td>
<td>0.70 (0.50-0.96)</td>
<td>0.03</td>
<td>20.9 (5.7-76.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>12.2 (4.2-35.3)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BMI, body mass index; CI, confidence interval; hs-CRP, high sensitivity C-reactive protein; OR, odds ratio; TSH, thyroid stimulating hormone.
RDW and impaired glucose control

it is elevated in many clinical settings, namely prehypertension, hypertension, colon cancer, thrombotic thrombocytopenic purpura (TTP), pregnancy, stroke, and inflammatory bowel diseases (IBDs)\textsuperscript{7,10,22,23}. As we found in this study, RDW was found to be correlated with inflammatory markers such as CRP\textsuperscript{24}. In addition to that, the independent predictors of high RDW for impaired glycemic control were found to be hs-CRP, BMI, and TSH, all of which are closely related to inflammation. These findings suggest that, beyond being an index parameter for erythrocyte size, an elevated RDW may reflect inflammation, indeed. The reason for the higher RDW levels in hyperglycemic patients may be oxidative stress, which causes cytoskeletal structural changes and endothelial dysfunction\textsuperscript{25,26}. In addition, RDW levels such as hs-CRP were found to be higher in diabetic patients than in prediabetic patients. Therefore, elevated RDW levels may even be associated with the degree of inflammation. Although reported routinely in CBC analysis, RDW is overlooked chiefly as an inflammatory marker and the association of RDW with inflammation needs to be more thoroughly investigated.

The first limitation of this study is the lack of subgroup analysis in the prediabetes group. The absence of other inflammatory and biochemical parameters such as IL-6, TNF-alpha, iron, vitamin B12 and folate is another limitation of our study.

Conclusions

RDW, a routine parameter of CBC analysis, is elevated in prediabetic and diabetic patients attributable to a subclinical inflammatory background.

Conflict of Interest

The authors declare that they have no conflict of interests.

Acknowledgements

None.

References

B-type natriuretic Peptide but not C-reactive protein are associated with higher red cell distribution width in patients with coronary artery disease. Int Heart J 2009; 50: 301-312.


