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# The Relationship Between Microvascular Complications in Type 2 Diabetes with Mean Platelet Volume, Red Blood Cell Distribution Width, Neutrophile Lymphocyte Ratio

## Tip 2 Diyabetlilerde Ortalama Trombosit Hacmi, Kırmızı Hücre Dağılım Genişliği ve Nötrofil/Lenfosit Oranı ile Mikrovasküler Komplikasyonlar Arasındaki İlişki

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**ABSTRACT Objective**: Diabetes mellitus (DM) is a complex syndrome caused by lack of insulin secretion and/or insulin resistance and emerges with high blood glucose levels, along with multiorgan involvement and characterized by micro/macro complications. Treatment process and complications of the disease are huge financial and moral problems for society as well as individuals. **Material and Methods:** Mean platelet volume (MPV), red cell distribution width (RDW), glycosylated hemoglobin (HbA1c), neutrophil and lymphocyte counts and ratio (Neutrophil lymphocyte ratio), microvascular complications (neuropathy, retinopathy and nepropathy) of 60 patiens diagnosed as diabetes and 60 healty individuals have been evaluated retrospectively. **Results:** In this study, we evaluated relationship between microvascular compliations with RDW, MPV, NLR. There was statistically significant difference between nephropathy with RDW, MPV, and NLR (respectively p: 0.008, p: 0.014 and p: 0.017). There was statistically significant difference between retinopathy with RDW and MPV (respectively p: 0.036 and p: 0.019), no statistically difference with NLR (p: 0.129). Within neuropathy, there was statistically significant difference between neuropathy with RDW and NLR (respectively p: 0.001 and p: 0.045), no relationship with MPV (p: 0.157). **Conclusion:** RDW, MPV, NLR values included in a simple laboratory test such as a hemogram are cost-effective parameters to demonstrate diabetic microvascular complications.

**Keywords:** Diabetes complications; diabetes mellitus, RDW, MPV, NLR

**ÖZET Amaç:** Diabetes mellitus (DM), insülin salınım eksikliği veya insülin direncinden kaynaklanan karmaşık bir sendromdur ve çoklu organ tutulumu ve mikro/makro komplikasyonlar ile karşılaşılmaktadır. Hastalığın tedavi süreci ve komplikasyonları toplum için olduğu kadar bireyler için de büyük maddi ve manevi sorunlardır. **Gereç ve Yöntemler:** Çalışmaya 60 sağlıklı birey ile 60 diyabet tanısı almış hasta alındı. Retrospektif olarak dosya taraması yapıldı ve hemoglobin, ortalama platelet hacmi (MPV), eritrosit dağılım genişliği (RDW), glikozillenmiş hemoglobin (HbA1c), nötrofil ve lenfosit sayıları ve oranı (NLR), mikrovasküler komplikasyonlar (nöropati, retinopati, nefropati) gibi bilgileri toplandı **Bulgular:** Diyabetik hastalarda mikrovasküler komplikasyonlar ile RDW, MPV, NLR arasındaki ilşkiyi incelediğimiz bu çalışmada RDW, MPV ve NLR ile nefropati arasında istatistiksel olarak anlamlı fark saptandı (sırası ile p: 0,008, p: 0,014 ve p: 0,017). Retinopati ile RDW ve MPV arasında istatistiksel anlamlı fark bulunurken (sırası ile p: 0,036 ve p: 0,019) NLR da ise anlamlı fark saptanmadı (p: 0,129). Nöropati ile RDW ve NLR arasında istatistiksel anlamlı fark bulunurken (sırası ile p: 0,001 ve p: 0,045) MPV de ise anlamlı fark saptanmadı (p: 0,157). **Sonuç:** Hemogram gibi basit bir laboratuar testinin içinde bulunan RDW, MPV, NLR değerleri diyabetik mikrovasküler komplikasyonları göstermede maliyet-etkin parametrelerdir.

**Anahtar Kelimeler:** Diyabet komplikasyonları; diabetes mellitus, MPV, RDW, NLR



Numerous biochemical, morphological and functional changes occur in the tissue and organs of diabetic patients. Acute complications may be at a life threatening level. Whereas, chronic complications

cause organ dysfunctions due to small and large vessels diseases that are developed in long term.[1]

Platelet volume is a marker of the function and activation of platelets and is measured as MPV with clinical hematology analyzers.[2] Several factors including endothelial dysfunction, impaired fibrinolysis, increased tendency to thrombosis and increased platelet activation-aggregation play a role in acceleration of atherosclerosis seen in diabetic patients. Platelet activity and aggregation capacity are essential in thrombogenesis and atherogenesis and this can be easily estimated with MPV. [3]Recently Neutrophil lymphocyte ratio (NLR) is recognized as a parameter giving information about the relationship between systemic inflammatory setting and physiological stress.[4] RDW elevation is also seen as the result of ineffective erythropoiesis due to chronic inflammation and neurohumoral activation. During inflammation, inflammatory cytokines suppress erythrocyte maturation, causing enter of juvenile erythrocytes to circulation and leading to increase in eritrosit heterogeneity.[5,6] There is a chronic inflammatory precess in diabetes which is known to play a role also in the development of diabetic complications. The objective of this study was to predict possible complications in diabetic patients under follow-up through evaluation of RDW, MPV, NLR from the outcomes of routine hemogram and thus, to demonstrate their applicability in microvascular complications of diabetes.

## MATERIAL AND METHODS

In this study, datas of 60 Type 2 DM patients who presented to Gaziantep University, Şahinbey Training and Research Hospital, Endocrinology outpatient clinic between December 2012 and December 2014, aged between 18-65 years and were using oral antidiabetics and 60 patients who presented to the endocrinology outpatient clinic due to any reason, but had no a known disease were retrospectively analyzed. Patients with a different chronic disease (coronary artery disease, hematologic malignancy, severe liver disease, severe renal failure), DM patients using insulin and smokers were excluded from the study. In the analyzes of blood samples collected from the patients and healthy persons; HbA1c data and MPV, RDW, NLR and platelet counts in the full blood count were recorded to the forms for both groups. The study was approved by Gaziantep University Hospital, Ethics Committee and written consents were received from all participants. Data obtained were evaluated with SPSS 22.0 statistical software.

In our study designed as screening; age, gender, hemoglobin, RDW, MPV, HbA1c, neutrophil count, lymphocyte count, mean platelet count, serum creatinine, full urine analysis, micro total protein (MTP) in 24-hour urine, electromyography (EMG) and fundoscopic examination outcomes were collected from the patient files. Glycemic control and the presence of microvascular complications (nephropathy, retinopathy and neuropathy) were studied.

Fundoscopic examination findings were assessed. At least two microaneurysm and/or retinal hemorrhage and/or retinal damage findings and laser photocoagulation were evaluated as retinopathy.

The presence of nephropathy was evaluated with amount of MTP in 24-h urine; the presence of at least 1 (+) proteinuira in patients without MTP in 24-h urine in their files (reasons such as leukocyturia that may lead to false positivity were ruled out). Patients with a MTP > 300 mg in 24-h urine were considered as diabetic nephropathy.

Diagnosis of neuropathy was assessed based on patients' clinical findings and EMG outcomes. Non-diabetic reasons of neuropathy (alcohol, uremic etc.) were ruled out in diabetic patients evaluated for neuropathy.

### STATISTICAL ANALYSIS

Normality of the continuous variables was controlled with Kolmogorov Smirnov test. Student-t test was used in comparison of two independent groups in normally distributed variables and Mann Whitney U test was used for non-normally distributed variables.

Correlations between categorical variables were tested using Chi-square test and correlations

between numerical variables were tested using correlation analysis. Logistic regression analysis was carried out with corrected Relative Risks (RR) and 95% confidence intervals. Data were expressed as number, percentage, mean ± standard deviation. Statistical analysis was performed using SPSS 22.0 statistical software. $p<0.05$ values were considered as statistically significant.

## RESULTS

Demographic and laboratory parameters of healthy and diabetic patients have shown in Table 1. A weak positive correlation was observed between age and RDW in the healthy control group ($r=0.372$, $p=0.003$). Taking this into account, the logistic regression model was applied in the patient and control groups by elimination effect of age, and reflection of the increase in the levels of RDW, MPV and NLR on diabetic complications was calculated. After eliminating effect of age, every 1 unit increase in RDW increases the risk for complications by 2.107 folds (RR=2.107, p=0.003).

Likewise, after eliminating effect of age, every 1 unit increase in MPV increases the risk for complications by 1.630 folds (RR=1.630, p=0.001).

Similarly, we applied the logistic regression model for NLR and age and found that, after eliminating effect of age, every 1 unit increase in NLR increases the risk for diabetic complications by 15.235 folds (RR=15.235, p=0.001).

The correlation between the existing complicatiosn and RDW, MPV and NLR rates are shown in Table 2. Statistical significance was found between nephropathy and each three parameters. While a significant correlation was found between rethinopathy and RDW and MPV, there was a signif icant correlation between RDW and NLR in patients with neuropathy.

## DISCUSSION

In many epidemiological studies chronic inflammation has been shown to play an effective role in the pathogenesis of chronic diseases such as diabetes, metabolic syndrome and hypertension.[7,8]

**TABLE 1:** Demographic and laboratory parameters of healthy and diabetic individuals.

| | Groups | N | Mean | Standart Deviation | p |
|---|---|---|---|---|---|
| **Age** | Healthy | 60 | 43.68 | 12.44 | **0.001** |
| | Diabetic | 60 | 53.65 | 7.84 | |
| **HbA1c** | Healthy | 60 | 5.44 | 0.37 | **0.001** |
| | Diabetic | 60 | 7.75 | 1.12 | |
| **Hemoglobin** | Healthy | 60 | 14.15 | 1.44 | 0.348 |
| | Diabetic | 60 | 13.93 | 1.04 | |
| **Thrombocyte** | Healthy | 60 | 273100 | 64049 | 0.422 |
| | Diabetic | 60 | 264785 | 47944 | |
| **RDW** | Healthy | 60 | 13.44 | 0.67 | **0.001** |
| | Diabetic | 60 | 15.92 | 1.05 | |
| **MCV** | Healthy | 60 | 83.73 | 2.16 | **0.001** |
| | Diabetic | 60 | 86.34 | 2.36 | |
| **NLO** | Healthy | 60 | 1.37 | 0.32 | **0.001** |
| | Diabetic | 60 | 1.75 | 0.40 | |
| **MPV** | Healthy | 60 | 10.28 | 0.85 | **0.001** |
| | Diabetic | 60 | 12.59 | 0.97 | |

**TABLE 2:** The relationship between diabetic complication and RDW, MPV, NLR.

| | Neuropathy | N | Mean | Standart Deviation | P |
|---|---|---|---|---|---|
| **RDW** | No | 44 | 13.89 | 0.99 | **0.001** |
| | Yes | 16 | 14.95 | 0.81 | |
| **MPV** | No | 44 | 10.43 | 0.95 | 0.157 |
| | Yes | 16 | 11.46 | 1.16 | |
| **NLR** | No | 44 | 1.68 | 0.35 | **0.045** |
| | Yes | 16 | 1.94 | 0.49 | |
| | **Nephropathy** | | | | |
| **RDW** | No | 41 | 13.93 | 0.99 | **0.008** |
| | Yes | 19 | 14.70 | 1.03 | |
| **MPV** | No | 41 | 10.07 | 0.83 | **0.014** |
| | Yes | 19 | 12.37 | 1.03 | |
| **NLR** | No | 41 | 1.68 | 0.35 | **0.017** |
| | Yes | 19 | 1.90 | 0.47 | |
| | **Retinopathy** | | | | |
| **RDW** | No | 47 | 14.10 | 1.04 | **0.036** |
| | Yes | 13 | 14.43 | 1.10 | |
| **MPV** | No | 47 | 10.68 | 0.75 | **0.019** |
| | Yes | 13 | 11.26 | 1.02 | |
| **NLR** | No | 47 | 1.70 | 0.37 | 0.129 |
| | Yes | 13 | 1.93 | 0.47 | |

Cross sectional and prospective studies have demonstrated a positive correlation between the complications of Type 2 DM and CRP, IL-6 and white cell counts.[9] Inflammation due to chronic hyperglicemia may affect erythrocyte formation, half-life and deformation of erythrocyte and causes anisocytosis, increasing RDW level. In our study, mean RDW value of the patients included was 15.9% which is a level close to the upper limit of 11.4% and 16.6% that is accepted as the normal range. RDW is an objective parameter to evaluate the size of erythrocytes and can be studied during automatic blood count without creating additional costs. Inflammation causes increase in RDW by both impairing iron metabolism and reducing the response and production of erythropoietin (EPO) and shortening life span of erythrocytes.[10] Malandrino et al. found a significant correlation between high levels of RDW and macrovascular complications (MI, cardiac failure, stroke) in a study group consisted of diabetic and non-diabetic subjects. Again in the same study; there was a significant correlation between RDW and nephropathy in terms of microvascular complications, while no significant correlation was found between RDW and retinopathy.[11] In the present study, RDW was separately correlated with nephropathy, neuropathy and retinopathy. It has been shown in the literature that MPV is increased in diabetic patients and this could be resulted from the osmotic swelling due to increased blood glucose and some glucose metabolites.[12] In their study, Tschope et al. showed that erythrocytes were large and glycoprotein membrane receptors (CD62, CD63, thrombospondin) were much in diabetic patients and accordingly platelet activation was increased.[13] In our study, consistently with the previous studies we observed higher levels of MPV in diabetic patients compared with nondiabetic patients. Every 1 unit increase in MPV was found to increase the risk for DM by 1.63 folds and demonstrated the correlation between MPV and DM. However, besides studies showing a correlation between neuropathy and MPV, there are some studies reporting no correlation. In their study with 145 diabetic and 100 non-diabetic patients, Hekimsoy et al. found no statis tical significance between neuropathy and MPV. Increased NLR was found to be an indicator of poor prognosis in patients undergone cardiovascular intervention. Recently, mortality rate has been demonstrated to increase with elevations in NLR.[14,15] Similarly, in our study there was a significant correlation between NLR level and the complications of nephropathy and neuropathy. This correlation was not observed in retinopathy, might be explained by that patient groups were at a level treatable with oral antidiabetics and had no poorly controlled diabetes. The age ranges of healthy and diabetic individuals in the study are significantly different from each other. This age difference may have affected the outcomes.In this study, we tested evaluability of the complication risk through risk and easily accessible parameters including RDW, MPV and NLR that are found in outcomes of routine hemograms in diabetic patients under follow-up. Our study is needed to be supported with larger studies.

### *Conflict of Interest*

*Authors declared no conflict of interest or financial support.*

### *Authorship Contributions*

***Idea/Concept:*** *Mesut Özkaya;* ***Design:*** *Mesut Özkaya, Zeynel Sayıner;* ***Control/Supervision:*** *Mesut Özkaya;* ***Data Collection and/or Processing:*** *Selman Atakur;* ***Analysis and/or Interpretation:*** *Zeynel Sayıner, Ayten Eraydın;* ***Literature Review:*** *Selman Atakur;* ***Writing the Article:*** *Zeynel Sayıner;* ***Critical Review:*** *Mesut Özkaya;* ***References and Fundings:*** *Mesut Özkaya.*

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