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# Relationship between Newly Diagnosed Hypertensive Patients' Dipping Status and Mean Neutrophil Volume

## Yeni Tanı Almış Hipertansif Hastaların Dipping Durumu ile Ortalama Nötrofil Hacmi Arasındaki İlişki

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## Abstract

**Objective:** Little is known about the pathogenesis of essential hypertension (HT) despite the research conducted in this field. However, similar to other chronic diseases, an association has been shown between HT and inflammation. Thus, this study aimed to explore the association between an indicator of inflammatory response and mean neutrophil volume (MNV) in newly diagnosed hypertensive patients.

**Materials and Methods:** The medical records of patients newly diagnosed with HT were retrospectively reviewed. The control group comprised healthy persons with normal ambulatory blood pressure records. In accordance with their immersion status, newly diagnosed hypertensive patients were divided into two groups.

**Results:** This study included 222 patients: One hundred and forty four patients had HT, and one hundred and eighty-eight were normotensives. HT patients had significantly higher MNV than normotensive patients [144.1 (range: 136.1-152.1 vs.) 140.3 (range: 135.1-145.5), p=0.001], respectively. There were 51 patients with dipper HT and 53 patients with non-dipper HT in the hypertensive group. MNV was significantly higher in the non-dipper HT group [145.7 (range: 138.1-153.46) vs. 142.3 (range: 134.3-150.3), p=0.022], respectively. Multivariate regression analysis demonstrated that MNV [95% confidence interval (CI): 1,006-1,122, p=0.032] and pulse wave velocity (95% CI: 1,203-2,655, p=0.004) were independently correlated with the non-dipping status in newly diagnosed hypertensive patients.

**Conclusion:** Patients with newly diagnosed HT had higher MNV. In addition, increased MNV measurements were associated with non-dipper HT.

**Keywords:** Hypertension, mean neutrophil volume, pulse wave velocity, non-dipper hypertension, MNV

## Öz

**Amaç:** Hipertansiyon (HT) alanında yapılan araştırmalara rağmen esansiyel HT'nin patogenezi hakkında çok az şey bilinmektedir. Bununla birlikte, diğer kronik hastalıklara benzer şekilde, HT ve enflamasyon arasında bir ilişki gösterilmiştir. Bu nedenle, bu çalışma yeni tanı konmuş hipertansif hastalarda bir inflamatuar yanıt göstergesi ile ortalama nötrofil hacmi (MNV) arasındaki ilişkiyi incelemeyi amaçlamaktadır.

**Gereç ve Yöntemler:** Yeni HT tanısı almış hastaların tıbbi kayıtları retrospektif olarak incelendi. Kontrol grubu, kan basıncı kayıtları normal olan sağlıklı hastalardan oluşturuldu. Yeni tanı almış hipertansif hastalar, dipping durumlarına göre iki gruba ayrıldı.

**Bulgular:** Bu çalışmaya 222 hasta dahil edildi; 144 hasta yeni HT tanılı, 188 hastada ise normotansif olarak değerlendirildi. Hipertansif hastalar, normotansif hastalardan istatistiki olarak daha yüksek MNV'ye sahipti (sırasıyla 144,1, 140,3, p=0,001). Hipertansif grupta dipper HT'si olan 51 hasta ve non-dipper HT olan 53 hasta vardı. MNV, non-dipper HT grubunda anlamlı olarak daha yüksekti (sırasıyla 145,7, 142,3, p=0,022). Yeni tanı konmuş hipertansif hastaların multivariate regresyon analizinde, MNV'nin [%95 güven aralığı (GA): 1,006-1,122, p=0,032] ve nabız dalga hızının (%95 CI: 1.203-2.655, p=0,004) non-dipper HT ile ilişkili olduğunu göstermiştir.

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**Sonuç:** Yeni tanı konmuş hipertansif hastalarda MNV normotansif hastalara gore daha yüksekti. Ek olarak, artan MNV ölçümleri non-dipper HT ile ilişkiliydi.

**Anahtar Kelimeler:** Hipertansiyon, ortalama nötrofil hacmi, nabız dalga hızı, non-dipper hipertansiyon, MNV

## Introduction

Hypertension (HT) is a major public health concern worldwide, and is considered to be one of the primary causes of preventable deaths. HT has become a disease with high morbidity and mortality due to damage it causes to the peripheral arteries, heart, kidney, brain, and eyes, leading to a heavy burden on healthcare resources (1-4).

Despite numerous previous studies, the pathogenetic mechanisms underlying HT are unclear. However, it is known that, as in many other chronic diseases, inflammatory dysregulation is a common culprit in HT (5). An independent association between C-reactive protein, interleukin-6, or adhesion molecules, indicators of chronic low-grade inflammation and responsible for vascular changes in essential HT patients, has been demonstrated. Inflammation increases the proliferation of smooth muscle cells in vascular structures and, as a consequence, contributes to high blood pressure (6-9). At the same time, this inflammation causes increased adhesion molecule expression, immune cell activation, cytokine release, and oxidative stress leading to end-organ damage and progression (9).

Mean neutrophil volume (MNV) refers to the average size of circulating neutrophils and can be easily detected by automatic hematological cell analyzers (10,11). The presence of differences in MNV has been linked to an increased inflammatory response, and MNV is a marker of illness severity in various infectious illnesses, acute myocardial infarction, and trauma, regardless of neutrophil count (10,12-18).

There is insufficient knowledge about the possible relationship between MNV and BP variation in hypertensive and normotensive subjects. Thus, the purpose of this study was to compare MNV in normotensive and newly diagnosed hypertensive patients. Additionally, we examined the relationship between dipping status and MNV in patients who have recently been diagnosed with HT.

## Materials and Methods

### Study Population

Patients equipped with a 24 h ambulatory BP monitoring device with a pulse wave velocity (PWV) measurement feature for evaluating HT in the cardiology clinic between January 2013 and December 2018 were retrospectively involved in the study. This study was approved by the Ethics Committee of Selçuk University of Medical Sciences (decision no: 2018/407, date: 21.11.2018). After obtaining the ethics committee's approval, the patient's digital records, including demographic and clinical data, were collected. A patient was excluded if he or she had any of the following conditions in the past: diabetes mellitus, chronic renal disease (serum creatinine ≥1.5 mg/dL in men and ≥ 1.4 mg/dL in women), coronary artery disease, congenital heart disease, left ventricular systolic dysfunction (on echocardiography <50% ejection fraction), local or systemic infection, moderate or severe valvular disease, atrial fibrillation, obstructive sleep apnea, hyperthyroidism, known malignancy, anemia, hematological disorders, and taking regular medication for any reason (including antihypertensive drugs). Moreover, patients who had a recent history of infection or another acute inflammatory condition were excluded from this study.

### Blood Pressure Measurement Using Ambulatory BP Monitoring and Diagnosis of Hypertension

Ambulatory blood pressure was measured using the Mobil-O-Graph Arteriograph (I.E.M. GmbH, Stolberg, Germany) and was recorded on the computer. The arm cuff was placed on the non-dominant arm, and recordings were taken for 24 hours. During the study, blood pressure measurements were taken every 15 minutes throughout the day (from 7 a.m. to 10 p.m.) and every 30 minutes during the night (from 10 p.m. to 7 a.m.). The patients were instructed to continue their daily routine and remain calm when they felt the cuff swell. Mean systolic blood pressure (SBP), mean blood pressure and mean diastolic blood pressure (DBP) values were calculated for each patient during the daytime, nighttime, and 24 hours. In this study, HT was defined as having a 24-hour mean SBP greater than 130 mmHg and/or DBP greater than 80 mmHg, as well as a mean daytime SBP greater than 135 mmHg and/or DBP greater than 85 mmHg, according to the diagnostic criteria, and mean nighttime systolic BP greater than 125 and/or diastolic BP greater than 70 mmHg (19). Dipper HT was defined as an average decrease of more than 10% in the diastolic and SBP measurements taken at night. The ambulatory blood pressure measuring device automatically measured PWV (20).

### Blood Samples

Complete blood count and serum biochemistry values were recorded from blood samples taken during cardiology outpatient clinic admission. Hemoglobin, white blood cells, neutrophils, platelets, lymphocytes, and MNV were evaluated in the whole blood count. In addition, total cholesterol, high-density lipoproteins, low-density lipoproteins, serum creatinine, fasting blood glucose, and triglycerides were recorded in serum biochemistry.

The Beckman Coulter DXH 800 analyzer (Beckman Coulter, Fullerton, CA, USA) was used to measure MNV. The VCS technology of the Coulter DXH 800 analyzer measures the

volume conductivity and light scatter. The Coulter analyzer can assess the morphological changes seen in reactive neutrophils, including volume, conductivity, and scatter changes.

### Statistical Analysis

A statistical evaluation was conducted using SPSS 21.0 (SPSS Inc., Chicago, IL, USA). To test the compatibility of numerical variables with a normal distribution, the Kolmogorov-Smirnov test was employed. Descriptive statistics were presented as the arithmetic mean ± standard deviation and median (25-75%) for numerical variables, and as numbers and percentages for categorical data. In cases where the parametric test assumptions were met, the significance test of the difference between the two means was used to compare the two groups with respect to numerical variables. The Mann-Whitney U test was used if not. Differences between groups concerning categorical variables were analyzed using chi-square and Fisher's exact chi-square tests. Binary logistic regression analysis was used to detect factors associated with non-dipper HT. A value of $p<0.05$ was accepted as significant for all evaluations.

## Results

A total of two hundred and twenty-two patients were included in this retrospective study. HT was found in 104 patients (51.20±12.33 years, 57% male), while 118 patients (49.36±13.32 years, 55% male) were normotensive. The demographic, clinical, and laboratory parameters distribution was similar between the normotensive and hypertensive groups (Table 1). The hypertensive group exhibited higher 24-hour systolic and DBP compared to the normotensive

**Table 1. Comparison of demographic, clinical and laboratory characteristics of normotensive and hypertensive groups**

| Variable | Normotensive (n=118) | Hypertensive (n=104) | p-value |
|---|---|---|---|
| Age, year | 49.36±13.32 | 51.20±12.33 | 0.08 |
| Gender (male), n (%) | 55 (46.6%) | 57 (54.8%) | 0.223 |
| Body mass index (kg/m$^2$) | 28.70±3.71 | 28.42±2.43 | 0.972 |
| LDL, mg/dL | 125.06±38.41 | 134.21±31.89 | 0.271 |
| HDL, mg/dL | 43.62±9.41 | 45.64±9.13 | 0.176 |
| Triglyceride, mg/dL | 183.01±122 | 209±149.97 | 0.321 |
| Hgb, g/dL | 13.88±1.75 | 13.65±1.95 | 0.376 |
| White blood cell, 10$^3$/µL | 8.09±218 | 8.01±2.38 | 0.574 |
| Platelet, 10$^3$/µL | 246.75±69.45 | 261.07±66.04 | 0.191 |
| Glucose, mg/dL | 107.60±40.57 | 125.46±84.87 | 0.096 |
| Creatinine, mg/dL | 0.75±0.22 | 0.79±0.16 | 0.064 |
| BUN, mg/dL | 28.37±13.54 | 29.21±9.03 | 0.219 |
| $Na^+$, mEq/L | 137.91±2.56 | 138.17±2.63 | 0.482 |
| $K^+$, mEq/L | 4.32±0.40 | 4.40±0.45 | 0.296 |
| 24-h SBP, mmHg | 110.61±8.74 | 135.87±12.48 | <0.001 |
| 24-h DBP, mmHg | 69.00±7.79 | 83.39±9.52 | <0.001 |
| Daytime SBP, mmHg | 112.51±9.27 | 138.40±17.58 | <0.001 |
| Daytime DBP, mmHg | 70.12±8.39 | 85.14±10.29 | <0.001 |
| Nighttime SBP, mmHg | 107.15±8.39 | 130.89±10.18 | <0.001 |
| Nighttime DBP, mmHg | 66.89±8.11 | 81.57±8.87 | <0.001 |
| Pulse wave velocity, m/s | 6.77±1.69 | 8.62±1.49 | <0.001 |
| MNV | 140.3±5.21 | 144.07±7.99 | 0.001 |
| Neutrophil count | 5.13±219 | 5.56±6.03 | 0.659 |

LDL: Low-density lipoprotein, HDL: High-density lipoprotein, Hgb: Hemoglobin, BUN: Blood urea nitrogen, $Na^+$: Sodium, $K^+$: Potassium, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, MNV: Mean neutrophil volume

group (p<0.001). Furthermore, the hypertensive group demonstrated higher levels of PWV and MNV compared to the normotensive group in the study (p<0.001).

When 104 patients with HT were grouped as dipper and non-dipper, 53 patients (52.62±11.27 years, 56.6% male) were grouped as non-dipper HT and 51 patients (49.73±13.30 years, 52.9% male) as dipper HT. At baseline, the dipper and non-dipper groups demonstrated similar clinical and demographic characteristics (Table 2). Markers, such as hemogram, neutrophil, and thrombocyte, obtained from the complete blood count of the non-dipper and dipper HT groups were similar between the two groups (p>0.05). However, daytime and 24-hour diastolic and SBP were higher in the dipper group (p<0.05). In addition, PWV and MNV measurements were higher in the non-dipper group than in the dipper group (p=0.042, p=0.022, respectively).

In multivariate binary logistic regression analysis including Hb, night SBP, body mass index, MNV, and PWV, only MNV and PWV were related to non-dipper HT (p=0.032, p=0.004) (Table 3).

## Discussion

Previous studies have investigated multiple parameters of neutrophil activity in HT patients. The study's findings indicate that neutrophils could potentially modify the microenvironment in blood vessels by heightening oxidative stress, which in turn favors endothelial dysfunction. MNV has emerged as a new marker of activated neutrophils. To our knowledge, this is the first study to demonstrate that MNV levels are higher in hypertensive subjects than in normotensive subjects. Also, we found that MNV levels were significantly associated with dipper status in HT

**Table 2. Comparison of demographic, clinical and laboratory characteristics of dipper and non-dipper groups**

| Variable | Non-dipper (n=53) | Dipper (n=51) | p-value |
|---|---|---|---|
| Age, year | 52.62±11.27 | 49.73±13.30 | 0.174 |
| Gender (male), n (%) | 30 (56.6%) | 27 (52.9%) | 0.708 |
| Body mass index (kg/m$^2$) | 28.61±2.16 | 28.21±2.7 | 0.564 |
| LDL, mg/dL | 141.11±35.86 | 127.75±26.64 | 0.207 |
| HDL, mg/dL | 44.90±9.85 | 46.30±8.51 | 0.539 |
| Triglyceride, mg/dL | 194.43±153.51 | 221.13±144.48 | 0.151 |
| Hgb, g/dL | 13.73±1.82 | 13.56±2.10 | 0.887 |
| White blood cell, 10$^3$/µL | 7.65±2.13 | 8.38±2.58 | 0.101 |
| Platelet, 10$^3$/µL | 262.43±68.92 | 258.68±63.69 | 0.682 |
| Glucose, mg/dL | 142.84±113.19 | 109.52±4.65 | 0.374 |
| Creatinine, mg/dL | 0.88±0.28 | 0.79±0.19 | 0.127 |
| BUN, mg/dL | 36.44±19.27 | 30.41±11.60 | 0.163 |
| $Na^+$, mEq/L | 138.02±2.97 | 138.32±2.29 | 0.730 |
| $K^+$, mEq/L | 4.38±0.41 | 4.41±0.48 | 0.768 |
| 24-h SBP, mmHg | 133.03±11.85 | 138.82±12.55 | 0.007 |
| 24-h DBP, mmHg | 81.58±8.60 | 85.27±10.14 | 0.021 |
| Daytime SBP, mmHg | 133.03±19.06 | 144.19±13.86 | <0.001 |
| Daytime DBP, mmHg | 81.58±8.77 | 88.84±10.53 | <0.001 |
| Nighttime SBP, mmHg | 129.83±9.97 | 132.00±10.38 | 0.268 |
| Nighttime DBP, mmHg | 81.92±8.65 | 81.21±9.19 | 0.995 |
| Pulse wave velocity, m/s | 8.91±1.17 | 8.31±1.72 | 0.011 |
| MNV | 145.76±7.70 | 142.31±7.99 | 0.022 |
| Neutrophil count | 5.85±8.27 | 5.27±2.05 | 0.057 |

LDL: Low-density lipoprotein, HDL: High-density lipoprotein, Hgb: Hemoglobin, BUN: Blood urea nitrogen, $Na^+$: Sodium, $K^+$: Potassium, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, MNV: Mean neutrophil volume

Table 3. Binary logistic regression analysis to determine independent variables associated with non-dipper hypertension

| | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| Variable | OR | 95% CI | p-value | OR | 95% CI | p-value |
| PWV | 1,334 | 1,008-1,766 | 0.044 | 1,787 | 1,203-2,655 | 0.004 |
| MNV | 1,058 | 1,005-1,114 | 0.03 | 1,067 | 1,006-1,122 | 0.032 |
| Nighttime SBP | 0.979 | 0.942-1,017 | 0.279 | 0.953 | 0.904-1,005 | 0.077 |
| BMI | 1,071 | 0.9713-1,257 | 0.401 | 1,220 | 0.978-1,510 | 0.069 |
| Hgb | 1,045 | 0.850-1,286 | 0.676 | 1,268 | 0.978-1,644 | 0.073 |

PWV: Pulse wave velocity, MNV: Mean neutrophil volume, SBP: Systolic blood pressure, BMI: Body mass index, Hgb: Hemoglobin, CI: Confidence interval, OR: Odds ratio

patients.

It has been recognized that low-grade inflammation plays a very significant pathophysiological role in cardiovascular disease and HT (6,10). Inflammation is involved in many processes that may contribute to developing high blood pressure. Vascular inflammation plays a role in vascular remodeling by increasing the proliferation of smooth muscle cells. Neutrophils are essential cells in inflammation (12,13,15,21). MNV represents the average size of the circulating neutrophil population. Studies showed that changes in MNV values are related to increased inflammatory response and indicate disease severity in many early trauma and infectious diseases, regardless of neutrophil counts (10,12,14,16,18).

In previous studies, a significant increase in MNV in cardiovascular diseases and sepsis conditions has been shown. van Hout et al. (13) carried out a study including 373 patients with ST-elevation myocardial infarction (STEMI), stable coronary artery disease, and coronary artery disease, which showed that MNV was significantly increased in patients with myocardial damage. In the study by Buyukterzi et al., (22) 121 patients without ACS, stable coronary artery disease, and coronary artery disease were, and their findings showed that MNV increased significantly in ACS patients. In our study, MNV was statistically significantly higher in patients with HT (22).

It has been shown in previous studies that non-dipper HT is related to a worse cardiovascular prognosis than dipper HT (23). In a study involving 42,947 patients, non-dipper HT has been associated with age, obesity, diabetes mellitus, and overt cardiovascular or kidney disease (24). In addition, Cuspidi et al.'s (25) study showed that non-dipper HT is more frequently associated with target organ damage than dipper HT. A statistically significant difference was observed between non-dipper HT patients and dipper HT patients in our study, and increased MNV values were associated with non-dipper HT.

Although the strict definition of the patient population in our study and the exclusion of patient groups that may be associated with high MNV other than HT constitute the strength of this study, there are some limitations to our study. The main limitations of our study are the relatively small number of patients enrolled in this study and the lack of evaluation of their response to antihypertensive treatment. However, we should note that MNV is a marker that can be easily measured and is a significant inflammatory indicator that may be a guide for further studies.

## Conclusion

MNV was higher in hypertensive patients than normotensive patients. In addition, increased MNV measurements were associated with non-dipper HT. Our study results need to be supported by large-scale prospective studies showing long-term prognosis.

### Ethics

**Ethics Committee Approval:** This study was approved by the Ethics Committee of Selçuk University of Medical Sciences (decision no: 2018/407, date: 21.11.2018)

**Informed Consent:** Informed consent is not required.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Concept: M.S.A., M.U.Y., Design: M.S.A., M.U.Y., Data Collection or Processing: M.S.A., A.T., K.D., N.A., Analysis or Interpretation: M.S.A., M.U.Y., A.T., K.D., N.A., Literature Search: M.S.A., M.U.Y., B.B.A., Writing: M.S.A., M.U.Y., B.B.A.

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