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The Relationship between Red Blood Cell Distribution Width and Blood Pressure Reverse Dipping in Patients with Essential Hypertension: A Cross-Sectional Study

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Abstract

Objective: To investigate whether Red blood cell distribution width (RDW) is associated with blood pressure (BP) reverse-dipper pattern in hypertensive patients.

Design: Cross-sectional study.

Setting: Single center.

Participants: Patients with essential hypertensive were included in our study(n=718). The exclusion criteria included age <18 or >90 years, incomplete clinical data, night workers, diagnosed as secondary hypertension, under antihypertensive treatment, intolerance for the 24 hours ambulatory blood pressure monitoring (ABPM), BP reading success rate < 70%.

Measurement: Physical examination and ABPM was performed for all patients in our study. The value of RDW was measured using an automated hematology analyzer.

Statistical methods: The distribution of RDW in hypertensive patients among different circadian BP pattern groups was analyzed using analysis of variance (ANOVA). Multinomial logistic regression was applied to explore the associations of RDW and other relevant variables with ABPM results.

Results: There was significantly increased RDW in reverse dippers (13.52±1.05) than dippers (13.23 ±1.00) of hypertension (p=0.007). Moreover, multinomial logistic regression analysis showed that RDW (OR 1.359, 95%CI 1.064-1.734, p=0.014), and diabetes mellitus (OR 0.415, 95%CI 0.251-0.685, p=0.001) were significantly different when comparing reverse-dipper BP pattern with dipper pattern. In addition to this, RDW was negatively correlated with the decline rate of nocturnal SBP (r=-0.123; p=0.001) and DBP (r=-0.111; p=0.003).

Conclusions: Our results suggest that RDW is associated with the reverse-dipper pattern of BP examined with 24h ABPM.

Strengths and limitations of this study

- This is the first study to evaluate red cell distribution width (RDW) levels in reverse-dipper pattern of blood pressure (BP).
- RDW is found to be strongly associated with reverse-dipper BP pattern.
- Our study had a cross-sectional design, whereas a longer period of prospective observation may provide more prognostic information.

- The subjects in our study are exclusively limited to northern Chinese patients from a single center.
- Specific markers of oxidative stress and inflammation were not investigated to reveal the exact mechanism of this association.

Introduction

Blood pressure (BP), as one of the vital signs, presents strong diagnostic and prognostic values, especially in hypotension and hypertension.[1] However, BP variations among different situations, activities and medical conditions are regulated by the nervous and endocrine systems. Resting BPs never represents the whole profile of circulation dynamics. Ambulatory blood pressure monitoring (ABPM), which offers us the average BP, variability, and circadian dipping status across a 24-hours (24-h) period, is considered the gold standard for diagnosis and the best predictor of the future end organ damage in patients with chronic hypertension.[2-4] The common circadian variation of BP presents a morning increase, small postprandial decline, more than 10% decrease in systolic blood pressure (SBP) and diastolic blood pressure (DBP) during sleep compared with daytime BP, and this is known as BP dipping.[5] Conversely, reverse-dipper hypertension, defined as an increase in SBP at night, was found to be predictor of cardiovascular events in hypertensive patients.[6-8]

Red blood cells (RBCs), formally known as erythrocytes, are one of the most important blood cells. RBCs deliver oxygen to the whole body via the circulatory system. The RBC distribution width (RDW), which is routinely detected in blood sample and widely used in hospital, describes the variation in RBC size.[9,10] RDW is usually elevated in conditions of increased erythrocyte destruction or ineffective erythropoiesis.[11] Clinical and experimental researches suggest that the assessment of RDW should be broadened far beyond the diagnostic value for anemia and it may

serve as an important biomarker in a variety of acute and chronic pathological conditions.[12,13] In recent years, RDW has been demonstrated to be independently associated with morbidity and mortality in cardiovascular and other various diseases, including coronary artery disease，heart failure，diabetes, stroke and venous thromboembolism.[14-18] It is reported that RDW is elevated in hypertensive patients compared with normotensives.[19] Additionally, studies have revealed that RDW levels is significantly higher in hypertensive patients with non-dipper pattern compared with dipper pattern.[20,21] However, no study has yet investigated any possible association between RDW and reverse-dipper BP pattern.

In this study, we aimed to determine whether RDW is elevated in hypertensive patients with reverse-dipper BP pattern compared with other circadian BP patterns. In addition, we tried to evaluate the potential association of RDW levels with the decline rate of nocturnal BP in all hypertensive patients.

Methods

Study population

We observed patients with essential hypertension continuously from January 2012 to June 2014. Data were extracted from our entire in-patient ABPM service database. Hypertension was diagnosed as SBP≥140mm Hg and/or DBP≥90 mm Hg in casual office recording, or daytime (or awake) SBP≥135 mmHg and/or DBP≥85 mm Hg, or night-time (or asleep) SBP ≥120 mm Hg and/or DBP≥70mm Hg in ABPM.[4] Diabetes mellitus (DM) was defined as fasting blood glucose≥126 mg/dl, casual blood glucose≥200 mg/dl, or 2-hour blood glucose≥200 mg/dl

during a 75g oral glucose tolerance test, or previous therapy for diabetes mellitus.[22] Smoking status was ascertained on the basis of self-reported history of cigarette smoking. The exclusion criteria included age <18 or >90 years, incomplete clinical data, night workers, diagnosed as secondary hypertension, under antihypertensive treatment, intolerance for the 24 hours ABPM, and BP reading success rate < 70%. Eventually, 718 hypertensive patients in total were included in our study. Physical examination and ABPM was performed for all patients. All patients were divided into three groups according to the presence of hypertension and circadian BP pattern as follows: dippers (average SBP and DBP decreased 10% to 20% of daytime level during sleep), non-dippers (<10% nocturnal BP fall) and reverse dippers (SBP nocturnal rise) .[23]

ABPM Assessment

All hypertension patients were evaluated with 24-hour ABPM using an oscillometric device (Spacelabs 90207; Spacelabs, Redmond, WA). The monitor was installed on the nondominant arm between 7:00 and $9:00_{AM}$ and removed 24 hours later. Frequency of recordings was made at every 15 minutes from $7:00_{AM}$ to $11:00_{PM}$ (diurnal BP values) and every 30 minutes from $11:00_{PM}$ to $7:00_{AM}$ (nocturnal BP values). Strenuous physical activity was discouraged for all patients during the monitoring period. Recordings were accepted only if more than 70% of the raw data was valid [4]. All ABPM recordings were manually edited by 2 individual physicians unaware of the objective and risk factors. Values of SBP <70 or >250 mm Hg, DBP <40 or >150 mm Hg, and heart rate<40 or >150 beats per minute were excluded from the recording. Fewer than 3% of the BP readings were rejected as artifacts on the basis of these criteria. The percent change in nocturnal blood pressure decline was calculated as follows: (mean diurnal BP - mean nocturnal

BP)/mean diurnal BP×100.

Biochemical Measurements

Baseline demographic, clinical and laboratory data were carefully recorded. Blood samples were collected from all patients in the morning after a fasting period of 12 h. RDW was measured by automated hematology analyzers according to the formula: RDW = (Coefficient of Variability of RBC /mean MCV) × 100.

Statistical Analysis

All statistical analyses were performed using the SPSS software package version 18.0(SPSS Inc., Chicago, IL, USA). Adequacy of all parameters to normal distribution, was tested by using Kolmogorov–Smirnov Test. Parametric tests were applied to data with normal distribution, while non-parametric tests were used to data without normal distribution. Descriptive statistics are presented as percentages for discrete variables and mean ± SD for continuous normally distributed variables. ANOVA test was used for statistical comparing of data that match with normal distribution. Multinomial logistic regression was applied to explore the associations among relevant variables with ABPM results. Bivariate correlation analysis was employed to examine the association between RDW and the decline rate of nocturnal BP. A calculated difference of $P<0.05$ was considered to be statistically significant.

The study protocol was approved by the ethics committee of the Second Affiliated Hospital, Xi'an Jiaotong University. All of the subjects consented to participate after been informed of the nature and purpose of the study.

Results

Baseline Characteristics of Patients

The clinical characteristics of patients in different groups according to circadian variations of BP were shown in Suppl. Table 1. In our study, a total of 171 patients (23.81%) had reverse-dipper BP pattern. Non-dipper pattern was observed in 370 hypertensive patients (51.53%) and dipper pattern in 177 patients (24.65%). Compared with dipper group, patients of reverse-dipper BP are older and more often suffering from diabetes mellitus, with a higher fasting blood glucose. There was significantly increased RDW in reverse dippers (13.52±1.05) than dippers (13.23±1.00) of hypertension (p=0.007).

Table 1 Characteristics of the Study Population by Dipping Status

Variable	Dipper	Nondipper	Reverse Dipper	*P* Value
Patients, n	177	370	171	
Age, y	56.08±14.50	58.79±13.54	64.91±11.97[ab]	<0.001
Male/female, n	100/77	200/170	90/81	0.761
Current smokers, n, %	42（23.73）	118（31.89）	55（32.16）	0.115
Diabetes mellitus, n, %	36（20.34）	95（25.68）	58（33.92）[a]	0.015
Fasting blood glucose, mmol/L	5.12±1.90	5.21±1.43	5.85±3.02[ab]	0.006
Triglycerides, mmol/L	2.23±1.90	1.82±1.25[a]	1.63±1.20[a]	<0.001
Total cholesterol, mmol/L	4.74±1.03	4.58±0.93	4.63±1.09	0.212
HDL-C, mmol/L	1.22±0.30	1.25±0.35	1.27±0.34	0.383
LDL-C, mmol/L	2.86±0.15	2.66±0.81[a]	2.72±0.88	0.050
VLD-C, mmol/L	0.70±0.57	0.67±0.53	0.65±0.58	0.728
24 h-SBP, ABPM, mm Hg	134.16±13.28	135.44±13.79	137.23±15.72[a]	0.127
SBP awakening, mm Hg	138.09±13.79	136.98±13.94	135.87±15.23	0.347
SBP bedtime, mm Hg	118.31±14.49	129.78±13.75[a]	142.52±16.84[ab]	<0.001
24 h-DBP, ABPM, mm Hg	79.44±11.79	80.00±10.08	79.11±10.04	0.628
DBP-awakening, mm Hg	82.53±10.82	81.02±10.10	78.86±9.98[ab]	0.004
DBP-bedtime, mm Hg	69.49±10.03	75.20±10.05[a]	79.94±10.93[ab]	<0.001

RDW	13.23±1.00	13.46±1.03[a]	13.52±1.05[a]	0.012

P for significant difference between the 3 groups. ABPM, ambulatory blood pressure monitoring; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; RDW, red blood cell distribution width; SBP, systolic blood pressure; VLD-C, very low-density lipoprotein cholesterol.

a) Indicated significance when comparing control with dipper group P<0.05.

b) Indicated significance when comparing control with nondipper group P<0.05

Association of RDW with Circadian BP Patterns

To explore the association of BP circadian patterns with RDW, a multinomial logistic regression analysis using stepwise selection process was performed in our study. It was discovered that RDW (OR 1.359, 95%CI 1.064-1.734, *p*=0.014), and diabetes mellitus (OR 0.415, 95%CI 0.251-0.685, *p*=0.001) were significantly different when comparing reverse-dipper BP pattern with dipper pattern (Table 2). Additionally, RDW (OR 1.313, 95%CI 1.056-1.632, *p*=0.014) and low-density lipoprotein cholesterol (LDL-C) (OR 0.764, 95%CI 0.623-0.938, *p*=0.001) were shown to be significantly different between non-dipper and dipper (Table 2).

Table 2 Multinomial Logistic Regression Analysis amoung Reverse Dipper, Nondipper, and Dipper

	Reverse Dipper vs Dipper		Nondipper vs Dipper		Reverse Dipper vs Nondipper		
Variable	OR(95% CI)	*P*	OR(95% CI)	*P*	OR(95% CI)	*P*	Global *P*
Diabetes mellitus	0.415(0.251~0.685)	0.001	0.652(0.417~1.018)	0.060	0.636(0.426~0.950)	0.027	0.002
Triglycerides	0.701(0.576~0.854)	＜0.001	0.836(0.739~0.947)	0.005	0.839(0.696~1.010)	0.064	＜0.001
LDL-C	0.838(0.659~1.064)	0.146	0.764(0.623~0.938)	0.010	1.096(0.889~1.351)	0.392	0.036
RDW	1.359(1.064~1.734)	0.014	1.313(1.056~1.632)	0.014	1.035(0.866~1.236)	0.707	0.019

Global *P* for significant difference between the 3 groups. CI, confidence interval; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; RDW, red blood cell distribution width.

Correlation between RDW Level and Decline Rate of Nocturnal BP

BP nocturnal decline is a physiological response to a range of neuroendocrinological factors. The nocturnal decline rate is a continuous variable, which also shows the BP dipping status. The smaller the decline rate is, the more abnormal the dipping status will be.[24] In order to further clarify the relationship between RDW level and the decline of nocturnal BP, bivariate correlation analysis was performed. Consistently, we found that RDW was negatively correlated with the decline rate of nocturnal SBP (r=-0.123; p=0.001) and DBP (r=-0.111; p=0.003) (Figure 1).

Discussion

As far as we know, hypertension is a common chronic condition and important risk factor for heart attacks, stroke and other vascular and target organ damages.[25] During the last 20 years, clinical investigations have demonstrated an important correlation between ABPM readings and prevalence and severity of cardiovascular events.[26,27] In the first published study in central Italy, ABPM was found to be the best predictor of atherosclerotic events.[28] Circadian BP variations were used to be divided into dipper (mean nocturnal BP drops 10mmHg or more than that in daytime) and non-dipper.[23,29] As a particular variant of non-dipper pattern, reverse-dipper pattern has a higher nighttime BP compared with daytime values, which was reported to be associated with higher incidence of cardiovascular events.[30] Moreover, in our previous studies, only reverse-dipper pattern of BP, instead of non-dipper pattern, is crucial in the early development of carotid plaque and lacuna infarction.[7,8]

Previously, the correlation between RDW and hypertension, especially non-dipper pattern of hypertension has been demonstrated in many studies.[31] Tanindiet *et al*. found that RDW was significantly higher in patients with prehypertension and hypertension than healthy subjects.[32]

Jithesh *et al.* also observed that both high-sensitive C-reactive protein (hs-CRP) and RDW levels were higher in the hypertensive patient group compared with the control group.[33] Furthermore, Wen showed that there was a close relationship between RDW levels and carotid artery atherosclerosis in patients with hypertension.[31] Besides, researchers reported that increased RDW levels were associated with higher BP levels according to a large community-based cohort studies.[34] Moreover, studies investigating RDW in patients with hypertension also reported that RDW levels is significantly increased in patients with non-dippers compared with dippers.[20,21]

RDW, a novel predictor of mortality, is strongly and independently associated with adverse outcomes in cardiovascular diseases.[35] The possible mechanism of the relationship between RDW and cardiovascular diseases is proposed to be chronic inflammation.[36] Lippi *et al.* described the association between RDW and inflammatory markers such as the erythrocyte sedimentation rate (ESR) and hs-CRP.[37] RDW elevation might reflect an underlying chronic inflammation, which may be due to the increased risk of cardiovascular diseases.[9] Additionally, oxidative stress was also revealed to play an important role in increased RDW in patients with cardiovascular diseases.[38] However, the detailed mechanism remains to be further investigated.

To our knowledge, this is the first study to evaluate RDW levels in reverse-dipper pattern of BP. In this study, we tried to determine whether any possible associations of circadian BP patterns with RDW levels existed in essential hypertension. We found that RDW levels were significantly higher in the reverse-dipper group than in dipper group. In addition, we carried out multiple logistic regressions and indicated that RDW is strongly associated with reverse-dipper hypertension. Furthermore, we found that the decline rate in BP from day to night was negatively correlated with RDW levels. Accordingly, the circadian decline rate in ABPM may also help to

evaluate the cardiovascular risks in hypertensive patients in clinics. As one of the most widely available laboratory data in the initial evaluation of hypertensive patients, RDW can thus be easily obtained to identify patients who are prone to reverse-dipper hypertension. Therefore, a more precise monitoring and management of BP could be administrated to prevent future cardiovascular risks.

Limitations

Several potential limitations should be noted. Firstly, our study had a cross-sectional design, whereas a longer period of prospective observation may provide more prognostic information. Secondly, the subjects in our study are exclusively limited to northern Chinese patients from a single center, therefore the conclusions should be drawn cautiously for other ethnic groups. Thirdly, although we demonstrated a significant association between elevated RDW and BP reverse-dipper pattern, we did not assess specific markers of oxidative stress and inflammation to reveal the exact mechanism of this association.

Conclusion

In conclusion, our study provides evidence that RDW may independently associate with the reverse-dipper pattern of BP and that potential prognostic value of RDW for cardiovascular diseases in people with hypertension would be usefully investigated.

Contributors GW, DS, JH A-qS and F-lZ contributed to the design of the work. DS and JH

collected the data. DS and QG wrote the manuscript. QG, YG, BY, L-yP were involved in the analysis and interpretation of data. GW, BY, A-qS and F-lZ reviewed the manuscript.

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Competing interests None declared.

Patient consent Obtained.

Ethics approval The study was approved by the ethics committee of the Second Affiliated Hospital, Xi'an Jiaotong University.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

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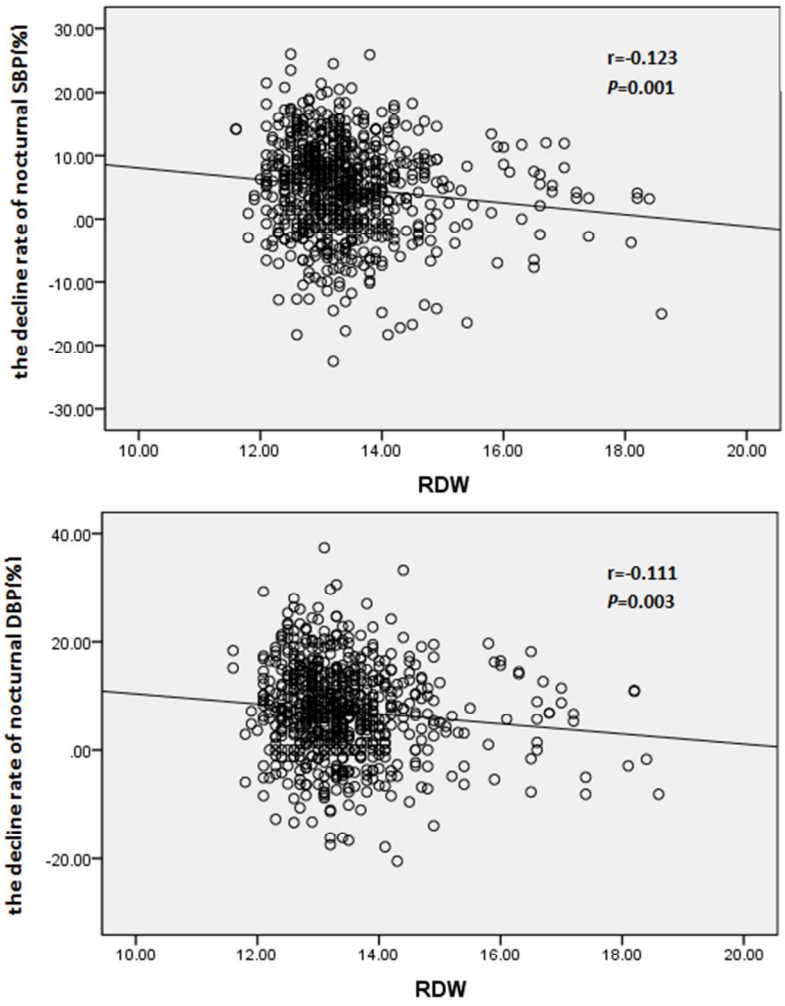

Figure 1.Correlation of RDW with the decline rate of nocturnal SBP and DBP.DBP, diastolic blood pressure; RDW, red blood cell distribution width; SBP, systolic blood pressure.

147x216mm (96 x 96 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
Outcome data	15*	Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses

1

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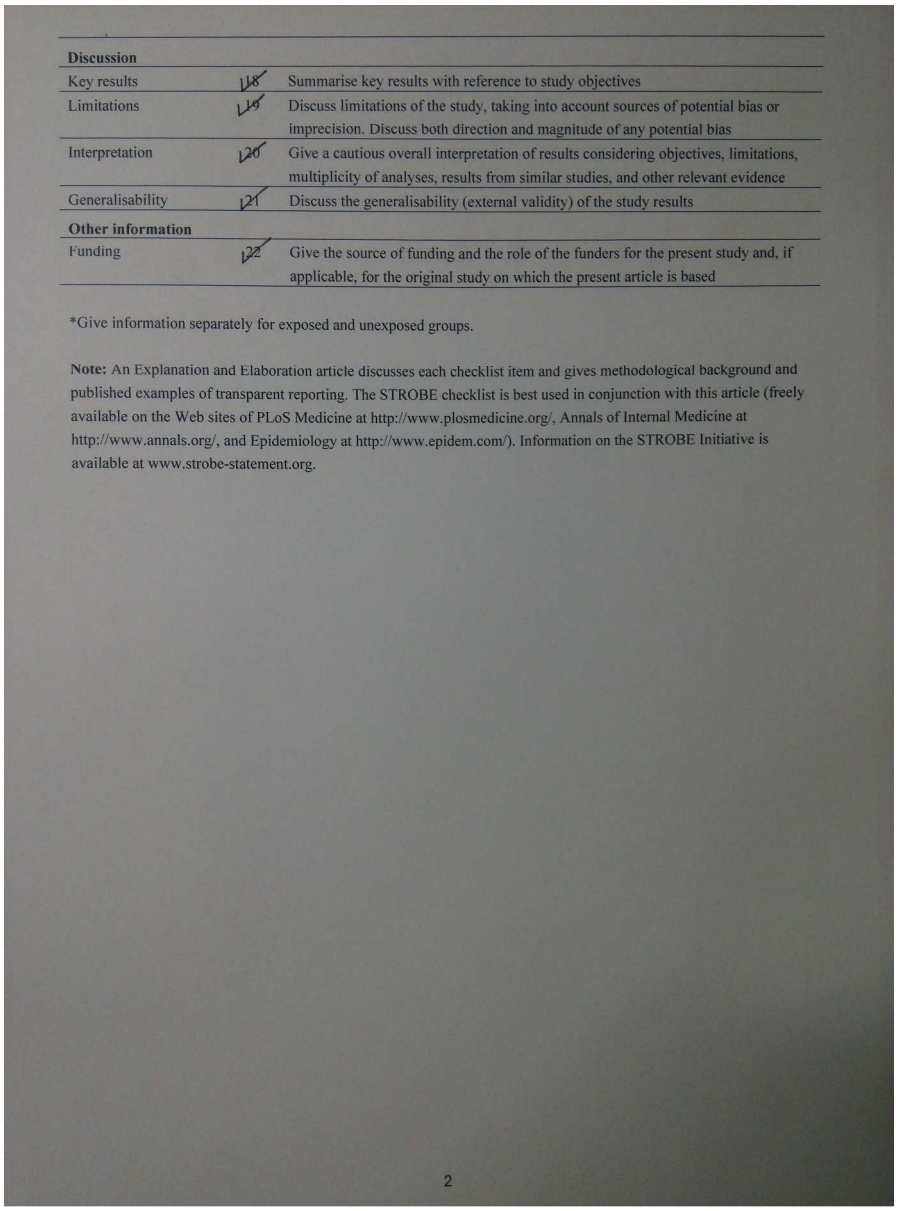

Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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The Relationship between Red Blood Cell Distribution Width and Blood Pressure Reverse Dipping in Patients with Essential Hypertension: A Cross-Sectional Study

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The Relationship between Red Blood Cell Distribution Width and Blood Pressure Reverse Dipping in Patients with Essential Hypertension: A Cross-Sectional Study

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Abstract

Objective: To investigate whether Red blood cell distribution width (RDW) is associated with blood pressure (BP) reverse-dipper pattern in hypertensive patients.

Design: Cross-sectional study.

Setting: Single center.

Participants: Patients with essential hypertensive were included in our study(n=718). The exclusion criteria included age <18 or >90 years, incomplete clinical data, night workers, diagnosed as secondary hypertension, under antihypertensive treatment, intolerance for the 24 hours ambulatory blood pressure monitoring (ABPM), BP reading success rate < 70%.

Measurement: Physical examination and ABPM was performed for all patients in our study. The value of RDW was measured using an automated hematology analyzer.

Statistical methods: The distribution of RDW in hypertensive patients among different circadian BP pattern groups was analyzed using analysis of variance (ANOVA). Multinomial logistic regression was applied to explore the associations of RDW and other relevant variables with ABPM results.

Results: There was significantly increased RDW in reverse dippers (13.52±1.05) than dippers (13.23 ±1.00) of hypertension (p=0.007). Moreover, multinomial logistic regression analysis showed that RDW (OR 1.359, 95%CI 1.064-1.734, p=0.014), and diabetes mellitus (OR 2.414, 95%CI 1.461~3.980, p=0.001) were significantly different when comparing reverse-dipper BP pattern with dipper pattern. In addition to this, RDW was negatively correlated with the decline rate of nocturnal SBP (r=-0.123; p=0.001) and DBP (r=-0.111; p=0.003).

Conclusions: Our results suggested that RDW might be associated with the reverse-dipper pattern of BP examined with 24h ABPM.

Strengths and limitations of this study

- This is the first study to evaluate red cell distribution width (RDW) levels in reverse-dipper pattern of blood pressure (BP).
- RDW is found to be strongly associated with reverse-dipper BP pattern.
- Our study had a cross-sectional design, whereas a longer period of prospective observation may provide more prognostic information.

- The subjects in our study are exclusively limited to northern Chinese patients from a single center.
- Specific markers of oxidative stress and inflammation were not investigated to reveal the exact mechanism of this association.

Introduction

Blood pressure (BP), as one of the vital signs, presents strong diagnostic and prognostic values, especially in hypotension and hypertension.[1] However, BP variations among different situations, activities and medical conditions are regulated by the nervous and endocrine systems. Resting BPs never represents the whole profile of circulation dynamics. Ambulatory blood pressure monitoring (ABPM), which offers us the average BP, variability, and circadian dipping status across a 24-hours (24-h) period, is considered the gold standard for diagnosis and the best predictor of the future end organ damage in patients with chronic hypertension.[2-4] The common circadian variation of BP presents a morning increase, small postprandial decline, more than 10% decrease in systolic blood pressure (SBP) during sleep compared with daytime BP, and this is known as BP dipping.[5] Conversely, reverse-dipper hypertension, defined as an increase in SBP at night, was found to be predictor of cardiovascular events in hypertensive patients. [6-8]

Red blood cells (RBCs), formally known as erythrocytes, are one of the most important blood cells. RBCs deliver oxygen to the whole body via the circulatory system. The RBC distribution width (RDW), which is routinely detected in blood sample and widely used in hospital, describes the variation in RBC size.[9,10] RDW is usually elevated in conditions of increased erythrocyte destruction or ineffective erythropoiesis.[11] Clinical and experimental researches suggest that the assessment of RDW should be broadened far beyond the diagnostic value for anemia and it may serve as an important biomarker in a variety of acute and chronic pathological conditions.[12,13] In

recent years, RDW has been demonstrated to be independently associated with morbidity and mortality in cardiovascular and other various diseases, including coronary artery disease，heart failure，diabetes, stroke and venous thromboembolism.[14-18] It is reported that RDW is elevated in hypertensive patients compared with normotensives.[19] Additionally, studies have revealed that RDW levels is significantly higher in hypertensive patients with non-dipper pattern compared with dipper pattern.[19,20] However, no study has yet investigated any possible association between RDW and reverse-dipper BP pattern.

In this study, we aimed to determine whether RDW is elevated in hypertensive patients with reverse-dipper BP pattern compared with other circadian BP patterns. In addition, we tried to evaluate the potential association of RDW levels with the decline rate of nocturnal BP in all hypertensive patients.

Methods

Study population

We observed patients with essential hypertension continuously from January 2012 to June 2014. Data were extracted from our entire in-patient ABPM service database. Hypertension was diagnosed as SBP≥140mm Hg and/or DBP≥90 mm Hg in casual office recording, or daytime (or awake) SBP≥135 mmHg and/or DBP≥85 mm Hg, or night-time (or asleep) SBP ≥120 mm Hg and/or DBP≥70mm Hg in ABPM.[4] Diabetes mellitus (DM) was defined as fasting blood glucose ≥126 mg/dl, casual blood glucose≥200 mg/dl, or 2-hour blood glucose≥200 mg/dl during a 75g oral glucose tolerance test, or previous therapy for diabetes mellitus.[21] Smoking status was

ascertained on the basis of self-reported history of cigarette smoking. The exclusion criteria included age <18 or >90 years, incomplete clinical data, night workers, diagnosed as secondary hypertension, under antihypertensive treatment, intolerance for the 24 hours ABPM, and BP reading success rate < 70%. Eventually, 718 hypertensive patients in total were included in our study. Physical examination and ABPM was performed for all patients. All patients were divided into three groups according to the presence of hypertension and circadian BP pattern as follows: dippers (average SBP decreased 10% to 20% of daytime level during sleep), non-dippers (<10% nocturnal SBP fall) and reverse dippers (SBP nocturnal rise) .[22]

ABPM Assessment

All hypertension patients were evaluated with 24-hour ABPM using an oscillometric device (Spacelabs 90207; Spacelabs, Redmond, WA). The monitor was installed on the nondominant arm between 7:00 and $9:00_{AM}$ and removed 24 hours later. Frequency of recordings was made at every 15 minutes from $7:00_{AM}$ to $11:00_{PM}$ (diurnal BP values) and every 30 minutes from $11:00_{PM}$ to $7:00_{AM}$ (nocturnal BP values). Strenuous physical activity was discouraged for all patients during the monitoring period. Recordings were accepted only if more than 70% of the raw data was valid.[4] All ABPM recordings were manually edited by 2 individual physicians unaware of the objective and risk factors. Values of SBP <70 or >250 mm Hg, DBP <40 or >150 mm Hg, and heart rate<40 or >150 beats per minute were excluded from the recording. Fewer than 3% of the BP readings were rejected as artifacts on the basis of these criteria. The percent change in nocturnal blood pressure decline was calculated as follows: (mean diurnal BP - mean nocturnal BP)/mean diurnal BP×100.

Biochemical Measurements

Baseline demographic, clinical and laboratory data were carefully recorded. Blood samples were collected from all patients in the morning after a fasting period of 12 h. RDW was measured by automated hematology analyzers according to the formula: RDW = (Coefficient of Variability of RBC /mean MCV) × 100.

Statistical Analysis

All statistical analyses were performed using the SPSS software package version 18.0(SPSS Inc., Chicago, IL, USA). Adequacy of all parameters to normal distribution, was tested by using Kolmogorov–Smirnov Test. Parametric tests were applied to data with normal distribution, while non-parametric tests were used to data without normal distribution. Descriptive statistics are presented as percentages for discrete variables and mean ± SD for continuous normally distributed variables. ANOVA test was used for statistical comparing of data that match with normal distribution. Multinomial logistic regression was applied to explore the associations among relevant variables with ABPM results. Bivariate correlation analysis was employed to examine the association between RDW and the decline rate of nocturnal BP. A calculated difference of $P<0.05$ was considered to be statistically significant.

The study protocol was approved by the ethics committee of the Second Affiliated Hospital, Xi'an Jiaotong University. All of the subjects consented to participate after been informed of the nature and purpose of the study.

Results

Baseline Characteristics of Patients

The clinical characteristics of patients in different groups according to circadian variations of BP were shown in Suppl. Table 1. In our study, a total of 171 patients (23.81%) had reverse-dipper BP pattern. Non-dipper pattern was observed in 370 hypertensive patients (51.53%) and dipper pattern in 177 patients (24.65%). Compared with dipper group, patients of reverse-dipper BP are older and more often suffering from diabetes mellitus, with a higher fasting blood glucose. There was significantly increased RDW in reverse dippers (13.52±1.05) than dippers (13.23±1.00) of hypertension (p=0.007).

Table 1 Characteristics of the Study Population by Dipping Status

Variable	Dipper	Nondipper	Reverse Dipper	*P* Value
Patients, n	177	370	171	
Age, y	56.08±14.50	58.79±13.54	64.91±11.97[ab]	<0.001
Male/female, n	100/77	200/170	90/81	0.761
Current smokers, n, %	42（23.73）	118（31.89）	55（32.16）	0.115
Diabetes, n, %	36（20.34）	95（25.68）	58（33.92）[a]	0.015
Fasting glucose, mmol/L	5.12±1.90	5.21±1.43	5.85±3.02[ab]	0.006
Triglycerides, mmol/L	2.23±1.90	1.82±1.25[a]	1.63±1.20[a]	<0.001
Total cholesterol, mmol/L	4.74±1.03	4.58±0.93	4.63±1.09	0.212
HDL-C, mmol/L	1.22±0.30	1.25±0.35	1.27±0.34	0.383
LDL-C, mmol/L	2.86±0.15	2.66±0.81[a]	2.72±0.88	0.050
VLD-C, mmol/L	0.70±0.57	0.67±0.53	0.65±0.58	0.728
24 h-SBP, ABPM, mm Hg	134.16±13.28	135.44±13.79	137.23±15.72[a]	0.127
SBP awakening, mm Hg	138.09±13.79	136.98±13.94	135.87±15.23	0.347
SBP bedtime, mm Hg	118.31±14.49	129.78±13.75[a]	142.52±16.84[ab]	<0.001
The decline rate of nocturnal SBP, %	13.99±3.15	5.16±2.82[a]	-5.08±4.39[ab]	<0.001
24 h-DBP, ABPM, mm Hg	79.44±11.79	80.00±10.08	79.11±10.04	0.628
DBP-awakening, mm Hg	82.53±10.82	81.02±10.10	78.86±9.98[ab]	0.004
DBP-bedtime, mm Hg	69.49±10.03	75.20±10.05[a]	79.94±10.93[ab]	<0.001
The decline rate of nocturnal DBP, %	15.77±5.97	7.22±5.36[a]	-1.51±6.58[ab]	<0.001
RDW	13.23±1.00	13.46±1.03[a]	13.52±1.05[a]	0.012

P for difference between the 3 groups. ABPM, ambulatory blood pressure monitoring; DBP,

diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; RDW, red blood cell distribution width; SBP, systolic blood pressure; VLD-C, very low-density lipoprotein cholesterol.

a) Indicated control with dipper group P<0.05.

b) Indicated control with nondipper group P<0.05

Association of RDW with Circadian BP Patterns

To explore the association of BP circadian patterns with RDW, a multinomial logistic regression analysis using stepwise selection process was performed in our study. Our univariate models included age, gender, smoking, diabetes, Triglycerides, HDL-C, LDL-C, VLD-C, 24h-SBP, 24h-DBP, SBP-awakening, SBP-bedtime, DBP-awakening, DBP-bedtime and RDW. The variables found significantly and accepted collinearity in univariate models was included in the multinomial analyses. It was discovered that RDW (OR 1.359, 95%CI 1.064-1.734, *p*=0.014), and diabetes mellitus (OR 2.414, 95%CI 1.461~3.980, *p*=0.001) were significantly different when comparing reverse-dipper BP pattern with dipper pattern (Table 2). Additionally, RDW (OR 1.313, 95%CI 1.056-1.632, *p*=0.014) and low-density lipoprotein cholesterol (LDL-C) (OR 0.764, 95%CI 0.623-0.938, *p*=0.001) were shown to be significantly different between non-dipper and dipper (Table 2).

Table 2 Multinomial Logistic Regression Analysis amoung Reverse Dipper, Nondipper, and Dipper

	Reverse Dipper vs Dipper		Nondipper vs Dipper		Reverse Dipper vs Nondipper		
Variable	OR(95% CI)	*P*	OR(95% CI)	*P*	OR(95% CI)	*P*	Global *P*
Diabetes	2.414(1.461~3.980)	0.001	1.534(0.982~2.397)	0.060	1.572(0.513~2.437)	0.027	0.002
Triglycerides	0.701(0.576~0.854)	＜0.001	0.836(0.739~0.947)	0.005	0.839(0.696~1.010)	0.064	＜0.001
LDL-C	0.838(0.659~1.064)	0.146	0.764(0.623~0.938)	0.010	1.096(0.889~1.351)	0.392	0.036
RDW	1.359(1.064~1.734)	0.014	1.313(1.056~1.632)	0.014	1.035(0.866~1.236)	0.707	0.019

Global *P* for significant difference between the 3 groups. CI, confidence interval; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; RDW, red blood cell distribution width.

Correlation between RDW Level and Decline Rate of Nocturnal BP

BP nocturnal decline is a physiological response to a range of neuroendocrinological factors. The nocturnal decline rate is a continuous variable, which also shows the BP dipping status. The smaller the decline rate is, the more abnormal the dipping status will be.[23] In order to further clarify the relationship between RDW level and the decline of nocturnal BP, bivariate correlation analysis was performed. Consistently, we found that RDW was negatively correlated with the decline rate of nocturnal SBP (r=-0.123; p=0.001) and DBP (r=-0.111; p=0.003) (Figure 1).

Discussion

As far as we know, hypertension is a common chronic condition and important risk factor for heart attacks, stroke and other vascular and target organ damages.[24] During the last 20 years, clinical investigations have demonstrated an important correlation between ABPM readings and prevalence and severity of cardiovascular events.[25,26] In the first published study in central Italy, ABPM was found to be the best predictor of atherosclerotic events.[27] Circadian BP variations were used to be divided into dipper (mean nocturnal SBP drops 10mmHg or more than that in daytime) and non-dipper.[22,28] As a particular variant of non-dipper pattern, reverse-dipper pattern has a higher nighttime SBP compared with daytime values, which was reported to be associated with higher incidence of cardiovascular events.[29] Moreover, in our previous studies, only reverse-dipper pattern of BP, instead of non-dipper pattern, is crucial in the early development of carotid plaque and lacuna infarction.[7,8]

Previously, the correlation between RDW and hypertension, especially non-dipper pattern of hypertension has been demonstrated in many studies.[30] Tanindiet *et al*. found that RDW was significantly higher in patients with prehypertension and hypertension than healthy subjects.[31] Jithesh *et al.* also observed that both high-sensitive C-reactive protein (hs-CRP) and RDW levels were higher in the hypertensive patient group compared with the control group.[32] Furthermore, Wen showed that there was a close relationship between RDW levels and carotid artery atherosclerosis in patients with hypertension.[30] Besides, researchers reported that increased RDW levels were associated with higher BP levels according to a large community-based cohort studies.[33] Moreover, studies investigating RDW in patients with hypertension also reported that RDW levels is significantly increased in patients with non-dippers compared with dippers.[19,20]

RDW, a novel predictor of mortality, is strongly and independently associated with adverse outcomes in cardiovascular diseases.[34] The possible mechanism of the relationship between RDW and cardiovascular diseases is proposed to be chronic inflammation.[35] Lippi *et al.* described the association between RDW and inflammatory markers such as the erythrocyte sedimentation rate (ESR) and hs-CRP.[36] RDW elevation might reflect an underlying chronic inflammation, which may be due to the increased risk of cardiovascular diseases.[9] Additionally, oxidative stress was also revealed to play an important role in increased RDW in patients with cardiovascular diseases.[37] However, the detailed mechanism remains to be further investigated.

To our knowledge, this is the first study to evaluate RDW levels in reverse-dipper pattern of BP. In this study, we tried to determine whether any possible associations of circadian BP patterns with RDW levels existed in essential hypertension. We found that RDW levels were significantly higher in the reverse-dipper group than in dipper group. In addition, we carried out multiple logistic

regressions and indicated that RDW is strongly associated with reverse-dipper hypertension. Furthermore, we found that the decline rate in BP from day to night was negatively correlated with RDW levels. Accordingly, the circadian decline rate in ABPM may also help to evaluate the cardiovascular risks in hypertensive patients in clinics. As one of the most widely available laboratory data in the initial evaluation of hypertensive patients, RDW can thus be easily obtained to identify patients who are prone to reverse-dipper hypertension. Therefore, a more precise monitoring and management of BP could be administrated to prevent future cardiovascular risks.

Limitations

Several potential limitations should be noted. Firstly, our study had a cross-sectional design, whereas a longer period of prospective observation may provide more prognostic information. Secondly, the subjects in our study are exclusively limited to northern Chinese patients from a single center, therefore the conclusions should be drawn cautiously for other ethnic groups. Thirdly, although we demonstrated a significant association between elevated RDW and BP reverse-dipper pattern, we did not assess specific markers of oxidative stress and inflammation to reveal the exact mechanism of this association.

Conclusion

In conclusion, our study provides evidence that RDW may independently associate with the reverse-dipper pattern of BP and that potential prognostic value of RDW for cardiovascular diseases in people with hypertension would be usefully investigated.

Contributors GW, DS, JH A-qS and F-lZ contributed to the design of the work. DS and JH collected the data. DS and QG wrote the manuscript. QG, YG, BY, L-yP were involved in the analysis and interpretation of data. GW, BY, A-qS and F-lZ reviewed the manuscript.

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Competing interests None declared.

Patient consent Obtained.

Ethics approval The study was approved by the ethics committee of the Second Affiliated Hospital, Xi'an Jiaotong University.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

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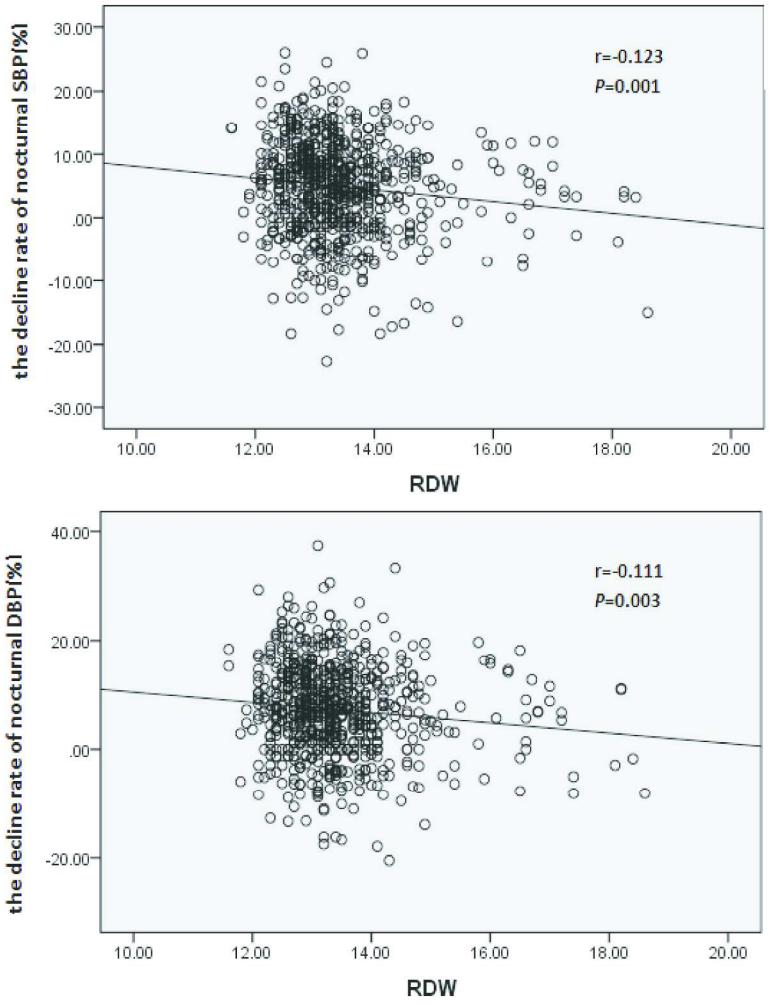

Figure 1.Correlation of RDW with the decline rate of nocturnal SBP and DBP.DBP, diastolic blood pressure; RDW, red blood cell distribution width; SBP, systolic blood pressure.

Correlation of RDW with the decline rate of nocturnal SBP and DBP
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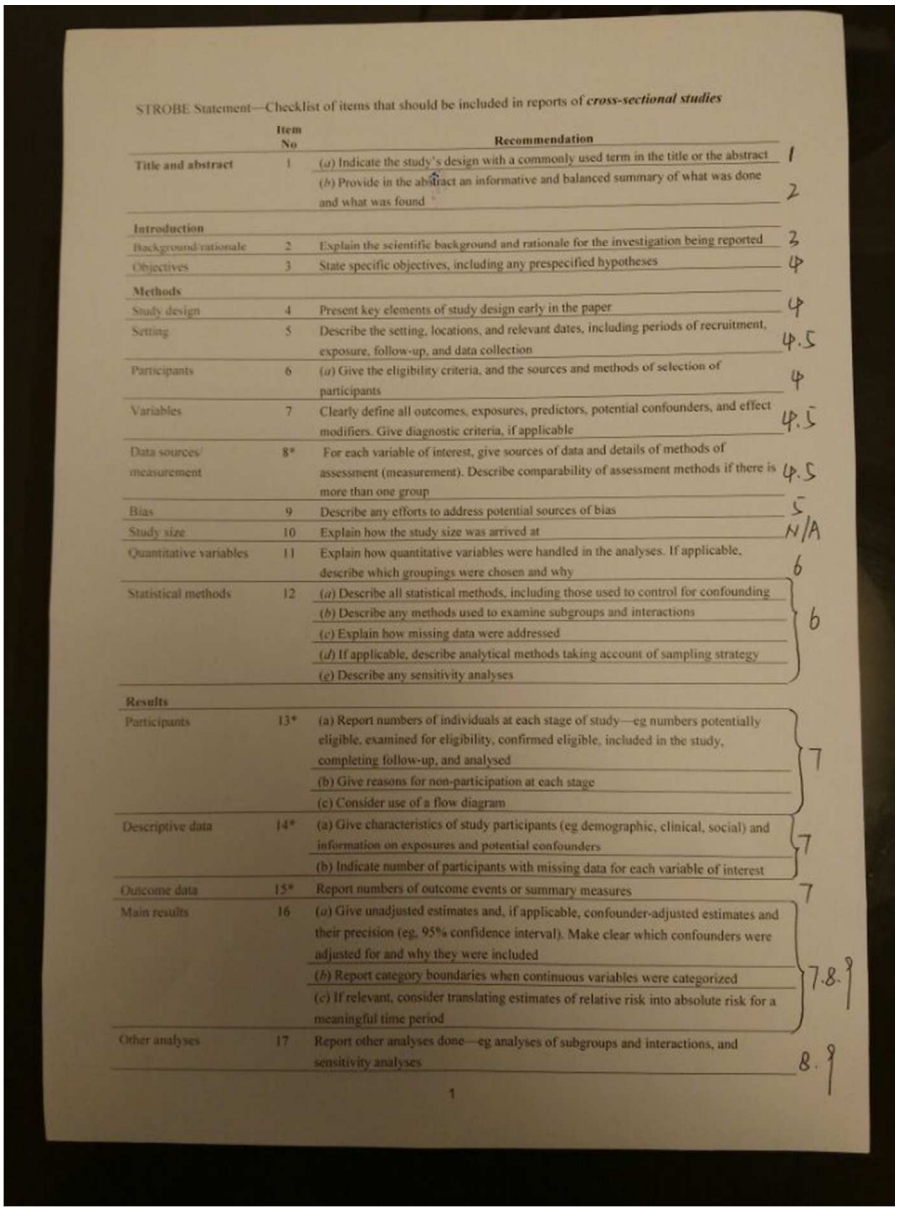

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4.5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4.5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4.5
Bias	9	Describe any efforts to address potential sources of bias	5
Study size	10	Explain how the study size was arrived at	N/A
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers of outcome events or summary measures	7
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7.8.9
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8.9

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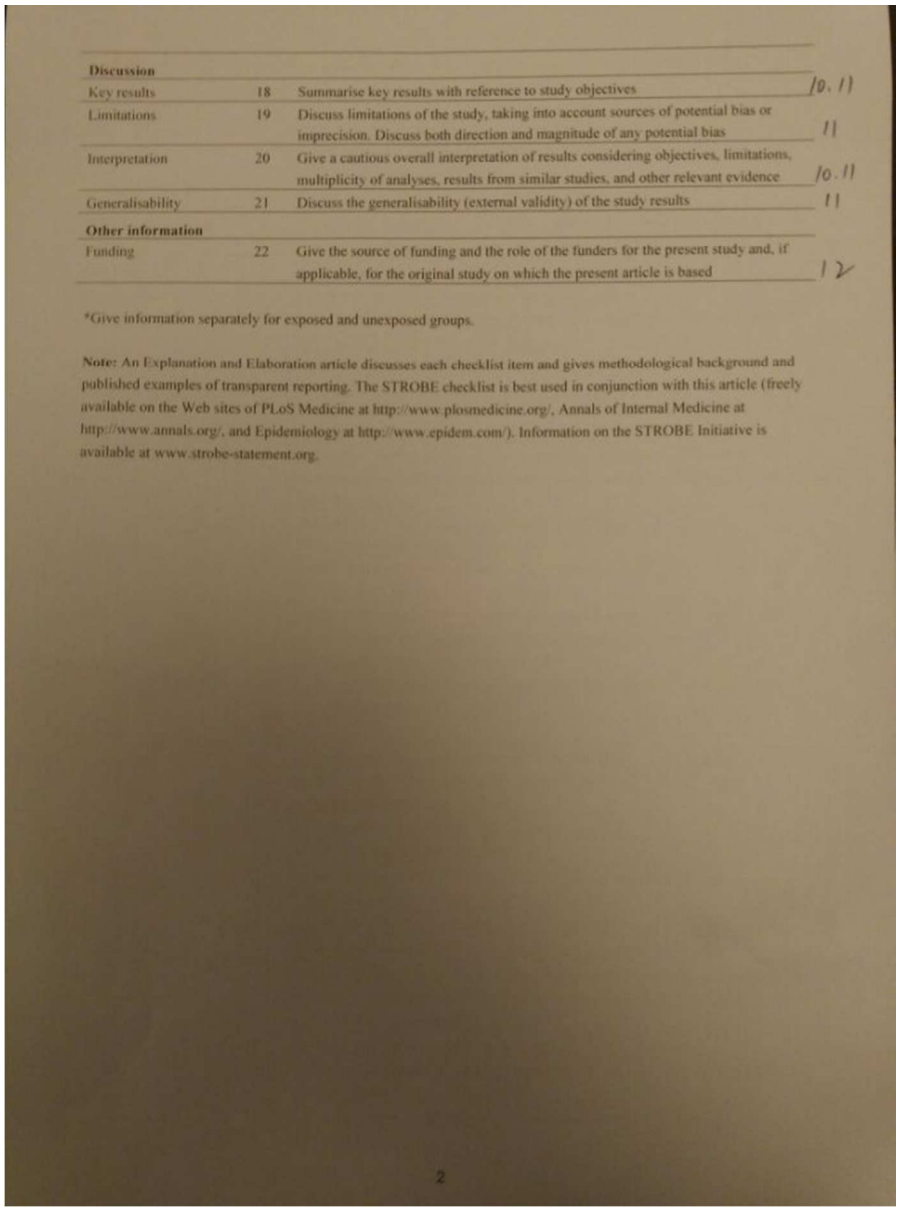

Discussion			
Key results	18	Summarise key results with reference to study objectives	10, 11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10, 11
Generalisability	21	Discuss the generalisability (external validity) of the study results	11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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The Relationship between Red Blood Cell Distribution Width and Blood Pressure Abnormal Dipping in Patients with Essential Hypertension: A Cross-Sectional Study

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The Relationship between Red Blood Cell Distribution Width and Blood Pressure Abnormal Dipping in Patients with Essential Hypertension: A Cross-Sectional Study

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Abstract

Objective: To investigate whether Red blood cell distribution width (RDW) is associated with blood pressure (BP) reverse-dipper pattern in hypertensive patients.

Design: Cross-sectional study.

Setting: Single center.

Participants: Patients with essential hypertensive were included in our study (n=708). The exclusion criteria included age <18 or >90 years, incomplete clinical data, night workers, diagnosed as secondary hypertension, under antihypertensive treatment, intolerance for the 24 hours ambulatory blood pressure monitoring (ABPM), BP reading success rate < 70%.

Measurement: Physical examination and ABPM was performed for all patients in our study. The value of RDW was measured using an automated hematology analyzer.

Statistical methods: The distribution of RDW in hypertensive patients among different circadian BP pattern groups was analyzed using analysis of variance (ANOVA). Multinomial logistic regression was applied to explore the associations of RDW and other relevant variables with ABPM results.

Results: There was significantly increased RDW in reverse dippers (13.52±1.05) than dippers (13.25 ±0.85) of hypertension (p=0.012). Moreover, multinomial logistic regression analysis showed that RDW (OR 1.325, 95%CI 1.037-1.692, p=0.024), and diabetes mellitus (OR 2.286, 95%CI 1.380~3.788, p=0.001) were significantly different when comparing reverse-dipper BP pattern with dipper pattern. However, there was no difference of RDW between non-dipper pattern and reverse dipper pattern (OR 1.036, 95%CI 0.867-1.238, p=0.693). In addition to this, RDW was still negatively correlated with the decline rate of nocturnal SBP (r=-0.113; p=0.003) and DBP (r=-0.101; p=0.007).

Conclusions: Our results suggested that RDW might associate with the abnormal dipper patterns of BP examined with 24h ABPM.

Strengths and limitations of this study

- This is the first study to evaluate red cell distribution width (RDW) levels in reverse-dipper pattern of blood pressure (BP).
- RDW is found to be associated with abnormal dipper BP patterns of either reverse dipping or non-dipping homogeneously.

- Our study had a cross-sectional design, whereas a longer period of prospective observation may provide more prognostic information.
- The subjects in our study are exclusively limited to northern Chinese patients from a single center.
- Specific markers of oxidative stress and inflammation were not investigated to reveal the exact mechanism of this association.

Introduction

Blood pressure (BP), as one of the vital signs, presents strong diagnostic and prognostic values, especially in hypotension and hypertension.[1] However, BP variations among different situations, activities and medical conditions are regulated by the nervous and endocrine systems. Resting BPs never represents the whole profile of circulation dynamics. Ambulatory blood pressure monitoring (ABPM), which offers us the average BP, variability, and circadian dipping status across a 24-hours (24-h) period, is considered the gold standard for diagnosis and the best predictor of the future end organ damage in patients with chronic hypertension.[2-4] The common circadian variation of BP presents a morning increase, small postprandial decline, more than 10% decrease in systolic blood pressure (SBP) during sleep compared with daytime BP, and this is known as BP dipping.[5] Conversely, reverse-dipper hypertension, which was used to be categorized as a variant of non-dipper, defined as an increase in SBP at night, was found to be predictor of cardiovascular events in hypertensive patients. [6-8]

Red blood cells (RBCs), formally known as erythrocytes, are one of the most important blood cells. RBCs deliver oxygen to the whole body via the circulatory system. The RBC distribution width (RDW), which is routinely detected in blood sample and widely used in hospital, describes the variation in RBC size.[9,10] RDW is usually elevated in conditions of increased erythrocyte destruction or ineffective erythropoiesis.[11] Clinical and experimental researches suggest that the

assessment of RDW should be broadened far beyond the diagnostic value for anemia and it may serve as an important biomarker in a variety of acute and chronic pathological conditions.[12,13] In recent years, RDW has been demonstrated to be independently associated with morbidity and mortality in cardiovascular and other various diseases, including coronary artery disease，heart failure，diabetes, stroke and venous thromboembolism.[14-18] It is reported that RDW is elevated in hypertensive patients compared with normotensives.[19] Additionally, studies have revealed that RDW levels is significantly higher in hypertensive patients with non-dipper pattern compared with dipper pattern.[19,20] However, it should be noticed that non-dipping pattern of BP in previous study included reverse dipping, which was recently regarded as an independent predictor for lacunar infarction, carotid plaque formation.[7,8] No study has yet investigated any possible association between RDW and reverse-dipper BP pattern.

In this study, we aimed to determine whether the level of RDW is elevated in hypertensive patients with reverse-dipper BP pattern compared with other circadian BP patterns. In addition, we tried to evaluate the potential association of RDW levels with the decline rate of nocturnal BP in all hypertensive patients.

Methods

Study population

We observed patients with essential hypertension continuously from January 2012 to June 2014. Data were extracted from our entire in-patient ABPM service database. Hypertension was diagnosed as SBP≥140mm Hg and/or DBP≥90 mm Hg in casual office recording, or daytime (or

awake) SBP≥135 mmHg and/or DBP≥85 mm Hg, or night-time (or asleep) SBP ≥120 mm Hg and/or DBP≥70mm Hg in ABPM.[4] Diabetes mellitus (DM) was defined as fasting blood glucose ≥126 mg/dl, casual blood glucose≥200 mg/dl, or 2-hour blood glucose≥200 mg/dl during a 75g oral glucose tolerance test, or previous therapy for diabetes mellitus.[21] Smoking status was ascertained on the basis of self-reported history of cigarette smoking. The exclusion criteria included age <18 or >90 years, incomplete clinical data, night workers, diagnosed as secondary hypertension, under antihypertensive treatment, intolerance for the 24 hours ABPM, and BP reading success rate < 70%. Eventually, 708 hypertensive patients in total were included in our study. Physical examination and ABPM was performed for all patients. All patients were divided into three groups according to the presence of hypertension and circadian BP pattern as follows: dippers (average SBP decreased 10% to 20% of daytime level during sleep), non-dippers (<10% nocturnal SBP fall) and reverse dippers (SBP nocturnal rise).[22]

ABPM Assessment

All hypertension patients were evaluated with 24-hour ABPM using an oscillometric device (Spacelabs 90207; Spacelabs, Redmond, WA). The monitor was installed on the nondominant arm between 7:00 and $9:00_{AM}$ and removed 24 hours later. Frequency of recordings was made at every 15 minutes from $7:00_{AM}$ to $11:00_{PM}$ (diurnal BP values) and every 30 minutes from $11:00_{PM}$ to $7:00_{AM}$ (nocturnal BP values). Strenuous physical activity was discouraged for all patients during the monitoring period. Recordings were accepted only if more than 70% of the raw data was valid.[4] All ABPM recordings were manually edited by 2 individual physicians unaware of the objective and risk factors. Values of SBP <70 or >250 mm Hg, DBP <40 or >150 mm Hg, and heart rate<40

or >150 beats per minute were excluded from the recording. Fewer than 3% of the BP readings were rejected as artifacts on the basis of these criteria. The percent change in nocturnal blood pressure decline was calculated as follows: (mean diurnal BP - mean nocturnal BP)/mean diurnal BP×100.

Biochemical Measurements

Baseline demographic, clinical and laboratory data were carefully recorded. Blood samples were collected from all patients in the morning after a fasting period of 12 h. RDW was measured by automated hematology analyzers according to the formula: RDW = (Coefficient of Variability of RBC /mean MCV) × 100.

Statistical Analysis

All statistical analyses were performed using the SPSS software package version 18.0(SPSS Inc., Chicago, IL, USA). Adequacy of all parameters to normal distribution, was tested by using Kolmogorov–Smirnov Test. Parametric tests were applied to data with normal distribution, while non-parametric tests were used to data without normal distribution. Descriptive statistics are presented as percentages for discrete variables and mean ± SD for continuous normally distributed variables. ANOVA test was used for statistical comparing of data that match with normal distribution. Multinomial logistic regression was applied to explore the associations among relevant variables with ABPM results. Bivariate correlation analysis was employed to examine the association between RDW and the decline rate of nocturnal BP. A calculated difference of $P<0.05$ was considered to be statistically significant.

The study protocol was approved by the ethics committee of the Second Affiliated Hospital,

Xi'an Jiaotong University. All of the subjects consented to participate after been informed of the nature and purpose of the study.

Results

Baseline Characteristics of Patients

The clinical characteristics of patients in different groups according to circadian variations of BP were shown in Suppl. Table 1. In our study, a total of 171 patients (24.15%) had reverse-dipper BP pattern. Non-dipper pattern was observed in 370 hypertensive patients (52.26%) and dipper pattern in 167 patients (23.59%). In addition, there were 10 extreme-dipper patients (more than 20% nocturnal SBP fall), who have been excluded from our study due to the insufficient sample size. Compared with dipper group, patients of reverse-dipper BP are older and more often suffering from diabetes mellitus, with higher fasting blood glucose. There was significantly increased RDW in reverse dippers (13.52±1.05) than dippers (13.25±0.85) of hypertension (p=0.012).

Table 1 Characteristics of the Study Population by Dipping Status

Variable	Dipper	Nondipper	Reverse Dipper	*P* Value
Patients, n	167	370	171	
Age, y	56.99±14.01	58.79±13.54	64.92±11.97[ab]	<0.001
Male/female, n	93/74	200/170	90/81	0.853
Current smokers, n, %	37（22.16）	118（31.89）	55（32.16）	0.052
Diabetes, n, %	35（20.96）	95（25.68）	58（33.92）[ab]	0.023
Fasting glucose, mmol/L	5.12±1.90	5.21±1.43	5.85±3.02[ab]	0.006
Triglycerides, mmol/L	2.20±1.89	1.82±1.25[a]	1.63±1.20[a]	0.001
Total cholesterol, mmol/L	4.74±1.05	4.58±0.93	4.63±1.09	0.235
HDL-C, mmol/L	1.23±0.31	1.25±0.35	1.27±0.34	0.450
LDL-C, mmol/L	2.86±1.06	2.66±0.81[a]	2.72±0.88	0.056
VLD-C, mmol/L	0.70±0.57	0.67±0.53	0.65±0.58	0.761

24 h-SBP, ABPM, mm Hg	133.98±13.21	135.44±13.79	137.23±15.72[a]	0.106
SBP awakening, mm Hg	137.73±13.65	136.97±13.94	135.87±15.23	0.478
SBP bedtime, mm Hg	118.68±14.57	129.78±13.75[a]	142.52±16.84[ab]	<0.001
the decline rate of nocturnal SBP, %	13.48±2.37	5.16±2.82[a]	-5.09±4.39[ab]	<0.001
24 h-DBP, ABPM, mm Hg	78.99±11.79	80.00±10.08	79.11±10.04	0.487
DBP-awakening, mm Hg	82.01±10.68	81.02±10.10	78.86±9.98[ab]	0.014
DBP-bedtime, mm Hg	69.49±10.17	75.20±10.05[a]	79.94±10.93[ab]	<0.001
the decline rate of nocturnal DBP, %	15.15±5.44	7.22±5.36[a]	-1.51±6.58[ab]	<0.001
RDW	13.25±0.85	13.46±1.03[a]	13.52±1.05[a]	0.026

P for difference between the 3 groups. ABPM, ambulatory blood pressure monitoring; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; RDW, red blood cell distribution width; SBP, systolic blood pressure; VLD-C, very low-density lipoprotein cholesterol.

a) Indicated control with dipper group P<0.05.

b) Indicated control with nondipper group P<0.05

Association of RDW with Circadian BP Patterns

To explore the association of BP circadian patterns with RDW, a multinomial logistic regression analysis using stepwise selection process was performed in our study. Our univariate models included age, gender, smoking, diabetes, Triglycerides, HDL-C, LDL-C, VLD-C, 24h-SBP, 24h-DBP, SBP-awakening, SBP-bedtime, DBP-awakening, DBP-bedtime and RDW. The variables found significantly and accepted collinearity in univariate models was included in the multinomial analyses. It was discovered that RDW (OR 1.325, 95%CI 1.037-1.692, *p*=0.024), and diabetes mellitus (OR 2.286, 95%CI 1.380~3.788, *p*=0.001) were significantly different when comparing reverse-dipper BP pattern with dipper pattern (Table 2). Additionally, RDW (OR 1.278, 95%CI 1.027-1.591, *p*=0.028) and low-density lipoprotein cholesterol (LDL-C) (OR 0.770, 95%CI 0.625-0.947, *p*=0.014) were shown to be significantly different between non-dipper and dipper (Table 2). However, there was no significant difference of RDW between non-dipper and reverse

dipper pattern (OR 1.036, 95%CI 0.867-1.238, p=0.693).

Table 2 Multinomial Logistic Regression Analysis amoung Reverse Dipper, Nondipper, and Dipper

	Reverse Dipper vs Dipper		Nondipper vs Dipper		Reverse Dipper vs Nondipper		
Variable	OR(95% CI)	*P*	OR(95% CI)	*P*	OR(95% CI)	*P*	Global *P*
Diabetes	2.286(1.380~3.788)	0.001	1.459(0.930~2.289)	0.100	1.567(1.050~2.339)	0.028	0.005
Triglycerides	0.713(0.585~0.870)	0.001	0.850(0.751~0.963)	0.011	0.839(0.697~1.011)	0.065	0.001
LDL-C	0.844(0.663~1.075)	0.169	0.770(0.625~0.947)	0.014	1.097(0.889~1.353)	0.388	0.047
RDW	1.325(1.037~1.692)	0.024	1.278(1.027~1.591)	0.028	1.036(0.867~1.238)	0.693	0.041

Global *P* for significant difference between the 3 groups. CI, confidence interval; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; RDW, red blood cell distribution width.

Correlation between RDW Level and Decline Rate of Nocturnal BP

BP nocturnal decline is a physiological response to a range of neuroendocrinological factors. The nocturnal decline rate is a continuous variable, which also shows the BP dipping status. The smaller the decline rate is, the more abnormal the dipping status will be.[23] In order to further clarify the relationship between RDW level and the decline of nocturnal BP, bivariate correlation analysis was performed. Consistently, we found that RDW was negatively correlated with the decline rate of nocturnal SBP (r=-0.113; p=0.003) and DBP (r=-0.101; p=0.007) (Figure 1).

Discussion

As far as we know, hypertension is a common chronic condition and important risk factor for heart attacks, stroke and other vascular and target organ damages.[24] During the last 20 years, clinical investigations have demonstrated an important correlation between ABPM readings and prevalence and severity of cardiovascular events.[25,26] In the first published study in central Italy, ABPM was found to be the best predictor of atherosclerotic events.[27] Circadian BP variations

were used to be divided into dipper (mean nocturnal SBP drops 10mmHg or more than that in daytime) and non-dipper (all other subjects).[22,28] As a particular variant of non-dipper pattern, reverse-dipper pattern has a higher nighttime SBP compared with daytime values, which was reported to be associated with higher incidence of cardiovascular events.[29] Moreover, in our previous studies, only reverse-dipper pattern of BP, instead of non-dipper pattern, is crucial in the early development of carotid plaque and lacuna infarction.[7,8]

Previously, the correlation between RDW and hypertension, especially non-dipper pattern of hypertension has been demonstrated in many studies.[30] Tanindiet *et al*. found that RDW was significantly higher in patients with prehypertension and hypertension than healthy subjects.[31] Jithesh *et al.* also observed that both high-sensitive C-reactive protein (hs-CRP) and RDW levels were higher in the hypertensive patient group compared with the control group.[32] Furthermore, Wen showed that there was a close relationship between RDW levels and carotid artery atherosclerosis in patients with hypertension.[30] Besides, researchers reported that increased RDW levels were associated with higher BP levels according to a large community-based cohort studies.[33] Moreover, studies investigating RDW in patients with hypertension also reported that RDW levels is significantly increased in patients with non-dippers compared with dippers.[19,20]

RDW, a novel predictor of mortality, is strongly and independently associated with adverse outcomes in cardiovascular diseases.[34] The possible mechanism of the relationship between RDW and cardiovascular diseases is proposed to be chronic inflammation.[35] Lippi *et al.* described the association between RDW and inflammatory markers such as the erythrocyte sedimentation rate (ESR) and hs-CRP.[36] RDW elevation might reflect an underlying chronic inflammation, which may be due to the increased risk of cardiovascular diseases.[9] Additionally,

oxidative stress was also revealed to play an important role in increased RDW in patients with cardiovascular diseases.[37] However, the detailed mechanism remains to be further investigated.

In this study, we tried to determine whether any possible associations of circadian BP patterns, especially reverse dipping, with RDW levels existed in essential hypertension. We found that RDW levels were significantly higher in the reverse-dipper group than in dipper group. In addition, we carried out multiple logistic regressions and indicated that RDW is strongly associated with reverse-dipper hypertension. Furthermore, we found that the decline rate in BP from day to night was negatively correlated with RDW levels. However, the difference of RDW level between non-dipper and reverse dipper patterns was not observed. Accordingly, we concluded that RDW might associate with both non-dipper and reverse dipper hypertension. Furthermore, the higher cardiovascular risk of patients with reverse-dipper pattern when compared to non-dipper pattern may not relate with a higher level of RDW.

Limitations

Several potential limitations should be noted. Firstly, our study had a cross-sectional design, whereas a longer period of prospective observation may provide more prognostic information. Secondly, the subjects in our study are exclusively limited to northern Chinese patients from a single center, therefore the conclusions should be drawn cautiously for other ethnic groups. Thirdly, we excluded 10 extreme dippers from the analysis in this study for their small sample size. Therefore, extreme dipping could be investigated in the future provided enough patients are recruited. Finally, although we demonstrated a possible association between elevated RDW and BP abnormal dipper patterns, their clinical significance requires further prospective investigation.

Conclusion

In conclusion, our study provides evidence that RDW may independently associate with the abnormal dipper pattern of BP and the higher cardiovascular risk of patients with BP reverse dipping comparing non-dipping may not due to the higher level of RDW.

Contributors GW, DS, JH A-qS and F-lZ contributed to the design of the work. DS and JH collected the data. DS and QG wrote the manuscript. QG, YG, BY, L-yP were involved in the analysis and interpretation of data. GW, BY, A-qS and F-lZ reviewed the manuscript.

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Competing interests None declared.

Patient consent Obtained.

Ethics approval The study was approved by the ethics committee of the Second Affiliated Hospital, Xi'an Jiaotong University.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

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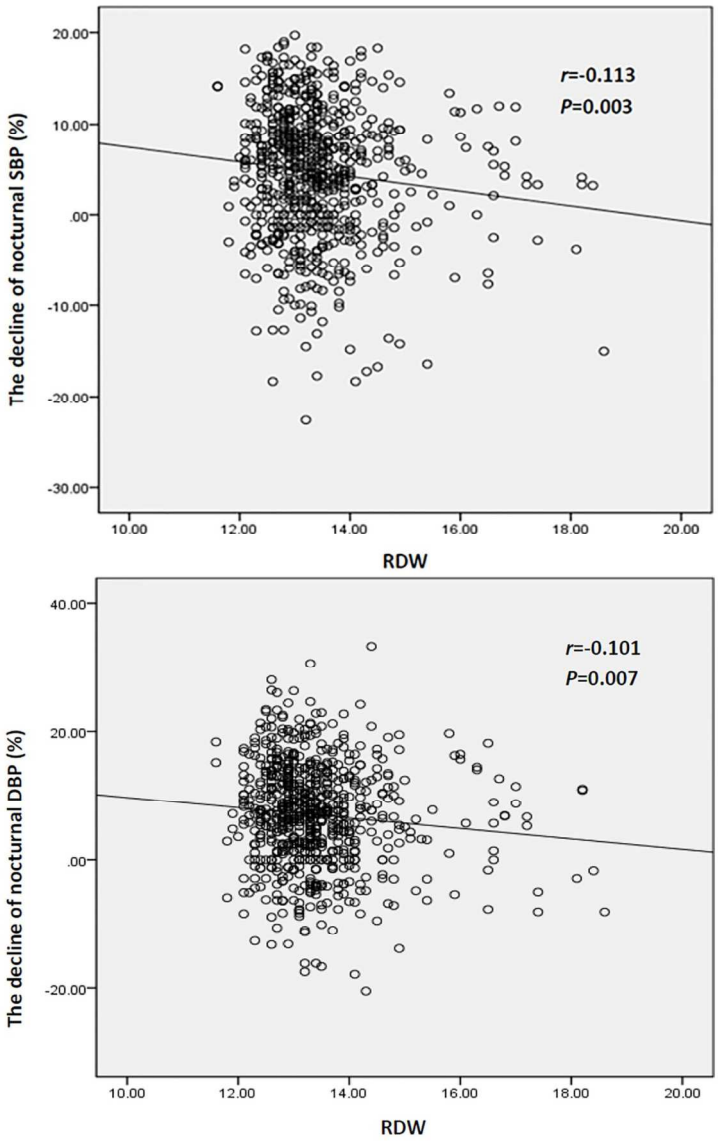

Figure 1. Correlation of RDW with the decline rate of nocturnal SBP and DBP. DBP, diastolic blood pressure; RDW, red blood cell distribution width; SBP, systolic blood pressure.

154x234mm (300 x 300 DPI)