

Original Article

Correlation between homocysteine levels and 24-h ambulatory blood pressure variability in Chinese hypertensive patients

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Abstract: Available evidence suggests that both homocysteine and blood pressure variability (BPV) are closely correlated with cardio-cerebrovascular disease. However, no previous study has addressed the correlation between homocysteine levels and 24-h ambulatory BPV. The aim of this study was to investigate the relationship between serum homocysteine levels and 24-h ambulatory BPV in Chinese hypertensive patients. This study consisted of 60 patients, divided into two groups according to the homocysteine level (high-level homocysteine group ($n = 15$), $\geq 15 \mu\text{M}$; low-level homocysteine group ($n = 45$), $< 15 \mu\text{M}$). The serum homocysteine, vitamin B12, and folate levels were measured. BPV was determined by 24-h ambulatory blood pressure (BP) monitoring. There were significant differences in 24-h systolic BP-standard deviation (SD), daytime systolic BP-SD, daytime diastolic BP-SD, nighttime systolic BP-SD, nighttime diastolic BP-SD, 24-h mean pulse pressure (PP)-SD, as well as serum vitamin B12 and folate levels between the high-level and low-level homocysteine groups. Multivariate linear regression analysis indicated that a high serum homocysteine level was an independent predictor of BPV and that the BPV increased with increasing homocysteine levels. The Spearman's rank correlation test indicated a positive correlation between the serum homocysteine level and the BPV index. In addition, hyperhomocysteinemia was an independent risk factor for BPV, and hypertensive patients with hyperhomocysteinemia had increased BPV. Thus, it is recommended to determine the homocysteine level and BPV index in patients with hypertension.

Keywords: Homocysteine, blood pressure variability, vitamin B12, folate

Introduction

Hypertension is a serious and increasing public health concern [1]. Globally, the overall prevalence of raised blood pressure (BP) in adults aged 25 and older was approximately 40% in 2008 [2]. In China, the prevalence of hypertension in urban and rural areas is reported to be 21.5% [3] and 22.81% [4] of the adult population, respectively. In addition, the incidence of hypertension in Chinese adults has increased during the last two decades [5]. Unfortunately, awareness, treatment, and control of hypertension in China is not satisfactory [6]. Hypertension is a well-known risk factor for cardiovascular disease. The adverse cardiovascular complications of hypertension not only depend on the magnitude of the BP elevation but may also depend on BP variability (BPV) [7]. Blood pres-

sure usually exhibits beat-to-beat, circadian, day-to-day, visit-to-visit, or seasonal variation. Clinical evidence has demonstrated that BPV contributes to the prognosis in hypertensive patients as well as the general population [8, 9].

Homocysteine, a sulfur-containing amino acid, is an intermediate in methionine metabolism. Elevated homocysteine levels are observed in hypertensive adults [10-12]. This coexistence of hyperhomocysteinemia and hypertension is described as H-type hypertension [13]. The prevalence of H-type hypertension is quite different mainly due to the various diagnostic standards of high homocysteine determination [14]. H-type hypertension is associated with arterial stiffness [15, 16], carotid disease [17], and cerebro-cardiovascular disease [18, 19]. At

a homocysteine level of $\geq 15 \mu\text{M}$, H-type hypertension was significantly correlated with the 6-month modified Rankin score in patients with acute ischemic stroke [20].

Despite increasing values of BPV and homocysteine levels both contributing to cardiovascular events, the association between homocysteine levels and BPV is unclear. The 24-h ambulatory BP monitor has been considered as a valuable tool for assessing short-term BPV. The aims of this study were to investigate the association between serum homocysteine levels and BPV by the use of 24-h ambulatory BP monitoring and to determine whether this relationship is independent of vitamin B12 and folate levels.

Methods

Study population

This cross-sectional study consisted of 60 patients (aged 40-80 years old) with mild-to-severe hypertension, who were hospitalized in the Department of Cardiology of the Second Hospital of Hebei Medical University from March to December, 2013. Hypertension was defined as systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg (Grade 1: $\geq 140/90$ mmHg; Grade 2: $\geq 160/100$ mmHg; Grade 3: $\geq 186/110$ mmHg), according to the Guidelines for the Prevention and Treatment of Hypertension in Chinese (2010 Revision) [21]. Patients with atrial fibrillation, psychiatric disorders, hyperpyrexia, secondary hypertension, or drug-induced hypertension (due to nonsteroidal anti-inflammatory drugs, licorice, amphetamine, oral contraceptives, steroids, cocaine, or cyclosporin A) were excluded. This study was approved by the Ethics Committee of the Second Hospital of Hebei Medical University. Written informed consent was obtained from all individuals who enrolled in the study.

Diagnostic criteria

Diabetes was diagnosed as a fasting plasma glucose level ≥ 7.0 mM and/or a 2-h postload glucose level ≥ 11.1 mM. Hyperlipidemia was defined as a serum level of total cholesterol ≥ 5.18 mM or triglycerides > 2.3 mM, according to the criteria of the Chinese Adult Dyslipidemia Prevention Guidelines [22]. Cerebrovascular disease was quantified by brain magnetic resonance imaging or magnetic resonance angiog-

raphy. Coronary heart disease was diagnosed by coronary computed tomography angiography or coronary arteriography. Hyperhomocysteinemia was defined as a homocysteine level $\geq 15 \mu\text{M}$ in the current study. According to the plasma homocysteine cutoff value of $15 \mu\text{M}$ [23], patients were divided into two investigation categories: a high-level homocysteine group ($n = 15$) or a low-level homocysteine group ($n = 45$).

Determination of BPV

BPV was determined by using a 24-h ambulatory BP monitor (SpaceLabs Medical Model 90207; Spacelabs Inc. USA). The ambulatory BP monitor was set to record the BP at 15-min intervals from 6:00 AM to 10:00 PM (daytime) and 30-min intervals from 10:00 PM to 6:00 AM (nighttime). The appropriate cuff was attached to the patients' nondominant arm. Records were considered appropriate when the number of successful readings exceeded 70% of the expected number of readings during 24 h and there were no breaks longer than 2 h [24]. Parameters of BPV were calculated as the standard deviation (SD) of ambulatory BP by using

$$\sigma = \sqrt{\frac{1}{N} \sum_{i=1}^N (x_i - \mu)^2}$$
, namely 24-h systolic BP-SD, 24-h diastolic BP-SD, 24-h mean pulse pressure (PP)-SD, daytime systolic BP-SD, daytime diastolic BP-SD, daytime mean PP-SD, nighttime systolic BP-SD, nighttime diastolic BP-SD, and nighttime mean PP-SD.

Measurement of serum levels of homocysteine, vitamin B12, and folate

A fasting venous sample of blood (5 mL) was collected into a tube containing 2 mL of ethylene diamine tetraacetic acid and then centrifuged at 2000 rpm/min. Finally, the serum was obtained. Serum levels of homocysteine were measured by using an enzyme-linked immunosorbent assay (IBL International GmbH; normal reference range of $5-15 \mu\text{M}$). Serum vitamin B12 and folate levels were assayed by a radioimmunoassay (Beijing Zhongxi Yuanda Science and Technology Co. Ltd.).

Statistical analysis

All statistical analyses were performed with SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Comparisons were made between the

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Table 1. Baseline demographics of the patients

Item	All patients (n = 60)	Low-level homocysteine group (n = 45)	High-level homocysteine group (n = 15)	p-value
Age (years), mean \pm SD	61.27 \pm 8.75	60.56 \pm 8.92	62.32 \pm 8.56	0.507
Female, n (%)	24 (40.00%)	17 (38.60%)	7 (43.80%)	0.721
Body mass index	25.92 \pm 4.36	25.86 \pm 4.12	26.07 \pm 4.78	0.870
Grade of hypertension, n (%)				
Grade 1	18 (30.00%)	12 (26.70%)	6 (40.00%)	0.652
Grade 2	25 (41.67%)	20 (44.40%)	5 (33.30%)	
Grade 3	17 (28.33%)	13 (28.90%)	4 (26.70%)	
Diabetes, n (%)	28 (46.67%)	23 (51.10%)	5 (33.30%)	0.232
Hyperlipidemia, n (%)	24 (40.00%)	20 (44.40%)	4 (26.70%)	0.224
Coronary heart disease, n (%)	42 (70.00%)	32 (71.10%)	10 (66.7%)	0.745
Cerebrovascular disease, n (%)	18 (30.00%)	14 (31.10%)	4 (26.7%)	0.745
Cigarette use, n (%)	25 (41.67%)	19 (42.20%)	6 (40.00%)	0.880

Table 2. Comparison of ambulatory blood pressure variability, vitamin B12, and folate levels

Item	Low-level homocysteine group (n = 45)	High-level homo- cysteine group (n = 15)	p-value
24-h systolic BP-SD	11.98 \pm 2.69	13.98 \pm 3.37*	0.041
Daytime systolic BP-SD	12.78 \pm 2.75	15.13 \pm 3.83*	0.032
Daytime diastolic BP-SD	8.23 \pm 2.91	11.10 \pm 3.81*	0.010
Nighttime diastolic BP-SD	7.83 \pm 3.38	11.46 \pm 5.31*	0.016
24-h mean PP-SD	8.43 \pm 1.79	10.87 \pm 3.17*	0.006
24-h diastolic BP-SD	9.17 \pm 2.24	9.86 \pm 3.06	0.062
Daytime mean PP-SD	9.26 \pm 2.26	10.68 \pm 3.28	0.058
Nighttime systolic BP-SD	12.56 \pm 2.21	13.92 \pm 6.18	0.074
Nighttime mean PP-SD	10.94 \pm 3.32	11.13 \pm 5.10	0.066
Serum folate (ng/mL)	10.50 \pm 2.91	7.56 \pm 4.39*	0.01
Serum vitamin B12 (pg/mL)	341.05 \pm 15.2	324.18 \pm 12.7*	0.0001

Data were expressed as the mean \pm SD; Compared with the low-level homocysteine group, * $P < 0.05$. BP = blood pressure; PP = pulse pressure; SD = standard deviation.

high-level and low-level homocysteine groups. Quantitative data were tested for normality of distribution using the Kolmogorov-Smirnov test. Continuous data were expressed as the mean \pm SD for normally distributed variables and examined using the independent sample t-test; otherwise, the nonparametric test was used. Multivariate stepwise logistic regression analysis was performed to identify potential risk factors independently associated with a high homocysteine level. Spearman's rank correlation test was used to analyze the correlation between the serum homocysteine levels

and parameters of BPV. A p -value < 0.05 was considered statistically significant.

Results

Baseline characteristics

A total of 60 hypertensive patients (36 men and 24 women), aged 40-80 years old, were included in this study. Of these, 18 patients had grade 1 hypertension, 25 had grade 2 hypertension, and 17 had grade 3 hypertension. BP was well-controlled (less than 130/80 mmHg) in all of the patients. Forty-two patients had coronary heart disease, 28 had diabetes, 24 had hyperlipidemia, and 18 had cerebrovascular

disease. The baseline characteristics of the patients are listed in **Table 1**.

Comparison of BPV parameters and vitamin B12 and folate levels

Table 2 lists the comparisons of BPV parameters as well as vitamin B12 and folate levels, based on the homocysteine levels. The 24-h systolic BP-SD, 24-h mean PP-SD, daytime systolic BP-SD, daytime diastolic BP-SD, and nighttime diastolic BP-SD were higher in the high-level homocysteine group compared with the

Table 3. Multivariate stepwise logistic regression analysis with homocysteine as the dependent variable

Variable	B regression coefficient	SE	Waldx	OR	p-value
24-h systolic BP-SD	1.967	1.086	5.76	8.82	0.014
Daytime systolic BP-SD	1.486	0.738	5.05	6.39	0.026
Nighttime systolic BP-SD	2.185	1.217	6.42	9.04	0.012
Nighttime mean PP-SD	1.003	0.387	3.36	3.92	0.041
Serum folate	1.076	0.687	4.72	4.96	0.034
Serum vitamin B12	2.358	1.349	6.60	10.53	0.005
Coronary heart disease	0.973	0.374	3.08	3.75	0.045

BP = blood pressure; PP = pulse pressure; OR = odds ratio; SD = standard deviation.

low-level homocysteine group (all p -values < 0.05). Serum vitamin B12 and folate levels were lower in the high-level homocysteine group than in the low-level homocysteine group (all p -values < 0.05).

Multivariate stepwise logistic regression analysis with homocysteine as the dependent variable

According to multivariate stepwise logistic regression analysis, the parameters of BPV, folate, vitamin B12, coronary heart disease, diabetes, hyperlipidemia, cerebrovascular disease, gender, and age were considered as independent variables and the serum homocysteine level was considered as a dependent variable. As shown in **Table 3**, multivariate stepwise logistic regression analyses indicated that 24-h systolic BP-SD, daytime systolic BP-SD, nighttime systolic BP-SD, nighttime mean PP-SD, serum folate, serum vitamin B12, and coronary heart disease were significantly associated with high homocysteine levels. Serum homocysteine risk increased by odds ratios (ORs) of 8.82 for 24-h systolic BP-SD, 6.39 for daytime systolic BP-SD, 9.04 for nighttime systolic BP-SD, 3.92 for nighttime mean PP-SD, 4.96 for serum folate, 10.53 for serum vitamin B12, and 3.75 for coronary heart disease.

Multivariate linear regression analysis with BPV as the dependent variable

The BPV parameters were selected as dependent variables. As shown in **Table 4**, multivariate linear regression analyses suggested that the homocysteine level was the important fac-

tor for daytime systolic BP-SD, daytime diastolic BP-SD, daytime mean PP-SD, nighttime systolic BP-SD, nighttime diastolic BP-SD, nighttime mean PP-SD, 24-h systolic BP-SD, 24-h diastolic BP-SD, and 24-h mean PP-SD. In other words, each unit increase in homocysteine level conferred approximately 0.2-0.5 greater variation of the BPV parameters.

Correlation analysis between serum homocysteine levels

and parameters of BPV

Using the Spearman correlation test, the serum homocysteine level was considered as an independent variable and parameters of BPV were considered as dependent variables. Spearman correlation analyses (**Table 5**) showed that the serum homocysteine levels were correlated with 24-h systolic BP-SD ($r = 0.15$, $P = 0.047$), 24-h mean PP-SD ($r = 0.38$, $P = 0.036$), daytime diastolic BP-SD ($r = 0.57$, $P = 0.028$), nighttime diastolic BP-SD ($r = 0.41$, $P = 0.032$), and daytime systolic BP-SD ($r = 0.44$, $P = 0.038$). A scatter plot of the correlation between the serum homocysteine levels and the BPV rate is shown in **Figure 1**.

Discussion

In the current study, we investigated the relationship between serum homocysteine levels and BPV in a small group of Chinese hypertensive patients. The results indicated that there were significant differences in 24-h systolic BP-SD, 24-h mean PP-SD, daytime systolic BP-SD, daytime diastolic BP-SD, nighttime diastolic BP-SD, serum vitamin B12 levels, and serum folate levels between the low-level and high-level homocysteine groups. BPV was higher among hypertensive patients with elevated homocysteine levels compared to hypertensive individuals with normal homocysteine levels. In addition, the serum homocysteine level was positively correlated with 24-h systolic BP-SD, daytime systolic BP-SD, daytime diastolic BP-SD, nighttime diastolic BP-SD, and 24-h mean PP-SD. Namely, a high level of homocysteine in hypertensive patients was significantly correlated with a high BPV rate. Multivariate

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Table 4. Multivariate linear regression analysis with blood pressure variability as the dependent variable

		Unstandardized Coefficients		Standardized Coefficients	t	p
		B	Std. Error	Beta		
Daytime systolic BP-SD	Constant	9.953	1.057		9.417	0.000
	Homocysteine	0.319	0.055	0.608	5.835	0.000
	Constant	7.628	0.668		11.415	0.000
Daytime diastolic BP-SD	Homocysteine	0.261	0.033	0.709	7.940	0.000
	Cerebrovascular disease	-1.956	0.932	-0.187	-2.099	0.040
Daytime mean PP-SD	Constant	7.578	0.505		15.016	0.000
	Homocysteine	0.232	0.026	0.759	8.889	0.000
Nighttime systolic BP-SD	Constant	9.591	0.617		15.549	0.000
	Homocysteine	0.290	0.032	0.767	9.100	0.000
Nighttime diastolic BP-SD	Constant	6.858	0.716		9.582	0.000
	Homocysteine	0.306	0.037	0.736	8.285	0.000
Nighttime mean PP-SD	Constant	6.797	0.719		9.449	0.000
	Homocysteine	0.288	0.037	0.713	7.738	0.000
24-h systolic BP-SD	Constant	6.649	0.918		7.240	0.000
	Homocysteine	0.496	0.047	0.808	10.443	0.000
24-h diastolic BP-SD	Constant	4.719	0.903		5.227	0.000
	Homocysteine	0.456	0.047	0.789	9.778	0.000
24-h mean PP-SD	Constant	5.106	0.834		6.120	0.000
	Homocysteine	0.424	0.043	0.790	9.825	0.000

BP = blood pressure; PP = pulse pressure; SD = standard deviation.

Table 5. Correlation analysis between serum homocysteine levels and parameters of blood pressure variability

	24-h systolic BP-SD	24-h mean PP-SD	Daytime diastolic BP-SD	Nighttime diastolic BP-SD	Daytime systolic BP-SD
p-value	0.047*	0.036*	0.028*	0.032*	0.038*
r	0.15	0.38	0.57	0.41	0.44

* $P < 0.05$; BP = blood pressure; PP = pulse pressure; SD = standard deviation.

linear regression analysis indicated that the homocysteine level was an important indicator of BPV and that the BPV increased with increasing homocysteine levels.

Studies investigating the relationship between serum homocysteine levels and BP have demonstrated that hypertensive patients with hyperhomocysteinemia correlate with a poor outcome [25, 26]. BPV reflects the actual blood fluctuation and forecasts damage to the target organs of the cardiovascular and cerebrovascular systems. Our study indicated that hyper-

tensive patients with hyperhomocysteinemia had a higher BPV index. However, the mechanisms underlying the relationship between homocysteine levels and BPV are largely unknown. Possible mechanisms might be that a high homocysteine level directly increases oxidative injury to the endothelium [27], leading to proliferation of smooth muscle cells and subsequently increasing the vascular wall thickness [28]. Meanwhile, accelerated collagen accumulation in the vascular endothelium alters the elastic properties of the vascular wall [29]. Elevated homocysteine levels also reduce the bioavailability of nitric oxide and endothelium-dependent vasodilatation [30]. In addition, homocysteine causes lipid peroxidation [31]. As both arteriosclerosis and endothelial dysfunction can contribute to BPV [32], these factors might be the link between elevated homocysteine levels and abnormal BPV.

Both homocysteine levels [33] and BPV [34] are independent predictors of cardiovascular events. Interrelations with homocysteine and

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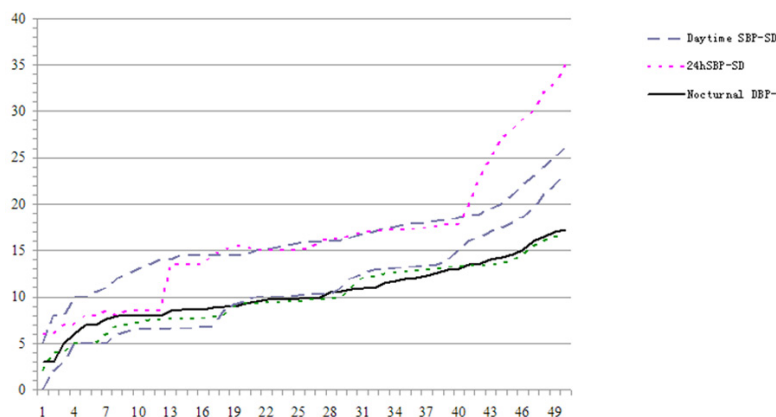


Figure 1. Scatter plot of the correlation between serum homocysteine levels and the index of blood pressure variability. The x-axis represents blood pressure variability. The y-axis represents serum homocysteine levels (μM).

BPV might augment the cardiovascular outcomes. High BPV increases the risk of target-organ damage both in hypertensive patients [35] and the general population [36]. A recently published meta-analysis [37] suggested that for each 1 mmHg increase of 24-h systolic BP, the hazard ratios of stroke, cardiovascular mortality, and total mortality were 1.02, 1.01, 1.12, and 1.03, respectively. Administration of amlodipine was effective for minimizing BPV compared with other antihypertensive agents [38]. It is important to consider reducing BPV when an antihypertensive agent is selected for hypertensive patients, which may improve cardio-cerebrovascular outcomes. In addition, our study demonstrated an inverse association between the serum levels of folate or vitamin B12 and homocysteine in hypertensive patients. The serum homocysteine levels may be influenced by different factors, such as regional climate and lifestyle. In particular, homocysteine metabolism requires folate and vitamin B12, and vitamin B12 and folate deficiencies can affect homocysteine levels [39, 40]. Hyperhomocysteinemia has been identified in more than half of hypertensive patients [41]. Therefore, folate, vitamin B12, and vitamin B6 supplementation appears to be the most economical method for managing the coexistence of hyperhomocysteinemia and hypertension.

This study has several limitations. First, a relatively small sample size of patients may limit the statistical power of this study. Second, the single-center hospitalized patient population

may have caused a selection bias. Third, this study did not determine the cause-effect relationship between elevated homocysteine levels and high BPV because of its cross-sectional design. Therefore, the conclusion that elevated homocysteine levels may have a significant effect on BPV is only speculative. Fourth, 24-h ambulatory BP monitoring only reflects the short-term BPV. The relationship between homocysteine levels and beat-to-beat, circadian, day-to-day, visit-to-visit, and seasonal variation of BP needs to be further studied. Finally, information regarding lifestyle factors, such as cigarette use and dietary habits, were not collected; these factors may influence the BP and homocysteine level.

Conclusions

This study suggests that homocysteine levels are an important indicator of BPV and that the BPV increases with increasing homocysteine levels. Thus, it is recommended to determine the homocysteine level and BPV index in patients with hypertension.

Disclosure of conflict of interest

None.

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