

BMJ Open Cross-sectional study on the relationship between the level of serum cystatin C and blood pressure reverse dipping in hypertensive patients

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ABSTRACT

Objective: To investigate the relationship between the level of serum cystatin C (s-CC) and reverse-dipper blood pressure (BP) pattern.

Design: Cross-sectional study.

Setting: Single centre.

Participants: A total of 718 hypertensive patients were eventually recruited from cardiac clinics between 2012 and 2014 in the Second Affiliated Hospital, Xi'an Jiaotong University. They were diagnosed as essential hypertension according to their casual office records of systolic blood pressure (SBP) and/or diastolic blood pressure (DBP). Patients were excluded if they were <18 or >90 years old, under antihypertensive treatment, night workers, suffering from acute stroke or myocardial infarction within the past 6 months, diagnosed as secondary hypertension, sleep apnoea or other sleep disorders, renal failure, cardiac failure, chronic obstructive pulmonary disease, women during pregnancy or intolerant to the ambulatory BP monitoring (ABPM).

Measurement: The selected patients were evaluated with 24 hours ABPM. Peripheral venous blood samples were collected to evaluate the s-CC levels by ELISA.

Methods: The distribution of hypertensive patients with different levels of s-CC among each circadian BP pattern group was analysed using analysis of variance. Multinomial logistic regression analysis was applied to explore the relationship between the relevant variables and ABPM results.

Results: S-CC level in reverse-dipper group (1.19 ±0.53 mg/L) was increased significantly when compared with dipper group (1.06±0.36 mg/L) (p=0.021). In addition, after multinomial logistic regression analysis, s-CC (OR 1.717; 95% CI 1.033 to 2.854; p=0.037) and diabetes (OR 2.313; 95% CI 1.401 to 3.821; p=0.01) were significantly different between the reverse-dipper group and dipper group. On the other hand, the decline rate of nocturnal SBP (r=-0.117; p=0.002) and DBP (r=-0.089; p=0.018) was negatively correlated with the s-CC level.

Conclusions: The s-CC level was significantly higher in the reverse-dipper group than the dipper group and that s-CC was associated with the reverse-dipper pattern of BP examined with 24 hour ABPM.

Strengths and limitations of this study

- The present study was the first to demonstrate significantly elevated level of serum cystatin C in hypertensive patients with reverse-dipper pattern of blood pressure.
- Our study was a cross-sectional study, long-term follow-up data were not provided.
- The patients in our study were recruited from a single centre in northern China.

INTRODUCTION

Renal function is conveniently evaluated using serum creatinine concentration in various clinical situations. However, serum creatinine is usually increased only when the glomerular filtration rate (GFR) is reduced by ~50%.¹ Serum cystatin C (s-CC) is considered to be a more precise marker for compromised GFR compared with serum creatinine, particularly for individuals with early kidney injury.²⁻³ Several studies have reported that s-CC also serves as an independent risk factor for target-organ damages and cardiovascular events in patients with essential hypertension.⁴⁻⁷ Therefore, it is believed that the monitoring of s-CC level is important for the clinical management of essential hypertension patients.

Hypertension is a major risk factor for the progression of cardiovascular and renal diseases,⁸ and blood pressure (BP) variations may provide additional clinical value.⁹⁻¹¹ Circadian BP patterns could be divided into dipper (10% to 20% systolic blood pressure (SBP) fall), extreme-dipper (>20% SBP fall), non-dipper (<10% SBP fall) and reverse-dipper (nocturnal SBP rise) in the light of the nocturnal fall of BP.¹²⁻¹³ Previous studies have reported that the incidence of target-organ damage in non-dipper group was increased significantly when compared

with dipper group.¹⁴ In addition, accumulating evidences have demonstrated that the reverse-dipper BP pattern, a variant of 'non-dipper', was strongly associated with cardiovascular injuries in chronic kidney disease.^{9 11 15} Furthermore, according to our study, BP reverse dipping was the real risk factor for carotid atherosclerosis and lacuna infarction, since the patients with non-dipper BP pattern failed to present the same risk.¹⁶

The relationships between s-CC and circadian variations of BP have been investigated before. A large cross-sectional study in California explored the relationship between kidney function measured with s-CC and each BP component using office BP measurements, and indicated that s-CC might associate with chronic kidney disease and hypertension.¹⁷ It was also reported earlier that the level of s-CC was higher in patients with 'non-dipper' hypertension compared with dippers.¹⁵ However, the association of s-CC with reverse-dipper BP pattern remains unknown. Therefore, we conducted this study to investigate the relationship between s-CC and reverse-dipper BP pattern. In addition, we tried to evaluate the potential association of s-CC levels with the decline rate of nocturnal BP in hypertensive patients.

METHODS

Design and participants

This was a single centre, cross-sectional study based on hypertensive individuals. During January 2012 to June 2014, a total of 718 participants were recruited. Data was extracted from the entire in-patient ambulatory BP monitoring (ABPM) service database in our hospital. Patients were excluded if they were <18 or >90 years old, under antihypertensive treatment, night workers, acute stroke or myocardial infarction within the past 6 months, sleep apnoea syndrome, secondary hypertension, could not tolerate the ABPM, pregnant female, arrhythmia, congestive heart failure, hepatic failure, kidney failure and chronic obstructive pulmonary disease. All the patients were referred to standard indications that have been shown to use ABPM for appropriate clinical circumstance.

We considered the participants to have clinical hypertension if their systolic BP (SBP) was >140mm Hg and/or diastolic blood pressure (DBP) was >90 mm Hg in casual office recording, or if their daytime (or awake) SBP was ≥ 135 mmHg and/or DBP was ≥ 85 mm Hg, or night-time (or asleep) SBP was ≥ 120 mm Hg and/or DBP was ≥ 70 mm Hg in ABPM.¹⁸ The decline rate of nocturnal BP was calculated as (daytime BP–night-time BP) $\times 100$ /daytime BP. The accepted normal value for SBP as 10–20% reduction in mean BP values at night compared with the daytime values.¹⁹ BP patterns of patients in our study were divided into dipper ($\geq 10\%$ and <20% SBP fall), non-dipper ($\geq 0\%$ and <10% SBP fall) and reverse dipper (nocturnal SBP rise), according to the range of the nocturnal SBP dip.¹³

Measurement

All the hypertensive patients were subjected to 24 hour ABPM using an oscillometric device (Spacelabs 90 207; Spacelabs, Redmond, Washington, USA). The arm cuff was fixed to the non-dominant upper limb between 07:00 and 09:00 and removed 24 hours later. BP was recorded every 15 min from 07:00 to 23:00 (daytime BP values) and every 30 min from 23:00 to 07:00 (nocturnal BP values). For each 24 hour ABPM, daytime BP and night-time BP means were calculated. Strenuous physical activity was discouraged in all patients, whose daily activities were comparable, during the monitoring period. All the ABPM recordings were reviewed by two individual physicians, who were not aware of the results and risk factors. Recordings were excluded from the analysis if: more than 30% of the raw data were missing;¹⁸ values of SBP <70 or >250 mm Hg, DBP <40 or >150 mm Hg and heart rate <40 or >150 bpm. Serum samples were obtained in the morning after a fasting period of 12 hour. S-CC was measured using an enzyme linked immunoassay kit (Biovendor Research and Diagnostic Products) at the central laboratory of Second Affiliated Hospital, Xi'an Jiaotong University.

Statistical analysis

Descriptive statistics are presented as percentages for discrete variables and mean \pm SD for continuous normally distributed variables. To compare ordinal and continuous normally distributed variables between subgroups of circadian BP and s-CC, χ^2 and analysis of variance (ANOVA) were employed, respectively. As multiple clinical variables exist, a multinomial logistic regression analysis using stepwise selection process was performed in our study to analyse the relationships between circadian BP patterns (dipper, non-dipper and reverse dipper) and related risk factors. Any variable with p values <0.1 after univariate analysis was brought into the multinomial logistic regression analyses. Correlation between s-CC level, as well as the decline rate of nocturnal SBP and DBP was assessed by bivariate correlation analysis. Linear regression analysis was also employed to test the relationship between nocturnal BP level and s-CC level. A calculated difference of p<0.05 was considered to be statistically significant. All the data was analysed using SPSS V.18.0.

RESULTS

Baseline characteristics

The clinical characteristics of the study population in different groups according to dipping status are shown in table 1. The average age of the 718 participants was 59.6 \pm 13.8 years and among them 54% were men. The mean s-CC level was 1.1 \pm 0.5 mg/L. The mean 24 hour SBP was 135.6 \pm 14.2 mm Hg and mean DBP 79.6 \pm 10.5 mm Hg. In our study, a reverse-dipper BP pattern was observed in 171 patients (23.8%) and a dipper pattern in 177 patients (24.7%). A total of 370

Table 1 Characteristics of the study population by dipping status

Variable	Dipper	Non-dipper	Reverse dipper	p Value
Patients, n	177	370	171	
WCH, n (15.60%)	50 (44.64%)	53 (47.32%)	9 (8.03%)	<0.001
SH, n (84.40%)	127 (20.95%)	317 (52.31)	162 (26.73%)	<0.001
Age, year	56.08±14.50	58.79±13.54	64.91±11.97†	<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)*	0.015
Fasting blood glucose, mmol/L	5.12±1.90	5.21±1.43	5.85±3.02*†	0.006
Triglycerides, mmol/L	2.23±1.90	1.82±1.25*	1.63±1.20*	<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*	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 hour Mean SBP, ABPM, mm Hg	134.16±13.28	135.44±13.79	137.23±15.72*	0.127
Mean SBP awakening, mm Hg	138.09±13.79	136.98±13.94	135.87±15.23	0.347
Mean SBP bedtime, mm Hg	118.31±14.49	129.78±13.75*	142.52±16.84*†	<0.001
24 hour Mean DBP, ABPM, mm Hg	79.44±11.79	80.00±10.08	79.11±10.04	0.628
Mean DBP-awakening, mm Hg	82.53±10.82	81.02±10.10	78.86±9.98*†	0.004
Mean DBP-bedtime, mm Hg	69.49±10.03	75.20±10.05*	79.94±10.93*†	<0.001
Cystatin C, mg/L	1.06±0.36	1.12±0.59	1.19±0.53*	0.062

*Indicated control with dipper group $p < 0.05$.

†Indicated control with non-dipper group $p < 0.05$.

ABPM, ambulatory blood pressure monitoring; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; SH, sustained hypertension (office BP $\geq 140/90$ with high ABPM measurements); VLD-C, very low-density lipoprotein cholesterol; WCH, white coat hypertension (office BP $\geq 140/90$ mm Hg with normal ABPM measurements).

hypertensive patients (51.5%) had non-dipper pattern. Compared with the dipper BP pattern, participants with reverse dipper were older, had a higher prevalence of diabetes, with a higher fasting glucose, triglycerides and significantly increased the level of s-CC (table 1). The s-CC level in the reverse-dipper group (1.19±0.53) was significantly higher when compared with dipper group (1.06±0.36) ($p=0.021$). However, there were no significant differences in other characteristics among the three groups.

Association of s-CC with reverse dipper BP pattern

In order to evaluate the association of different circadian BP patterns with s-CC level, a prescreening with ANOVA for multiple clinical variables was performed and it was found that diabetes, triglycerides, low-density lipoprotein cholesterol (LDL-C) and s-CC were significantly different between different BP dipping groups. After collinearity was assessed in the univariate models,

multinomial regression analyses were carried out which indicated that s-CC (OR 1.717, 95% CI 1.033 to 2.854; $p=0.037$) was significantly different between the BP reverse dipping and dipping groups (table 2). It was also discovered that diabetes (OR 2.313, 95% CI 1.401 to 3.821; $p=0.01$) and triglycerides (OR 0.704, 95% CI 0.578~0.858; $p < 0.001$) were significantly different between these two groups (table 2).

Correlation between s-CC level and the decline rate of nocturnal BP

In order to further investigate the relationship between the different circadian BP patterns and s-CC, we assessed the effect of the nocturnal drop of BP using the decline rate of nocturnal BP as a continuous variable, which also shows the BP dipping status. Bivariate correlation analysis was performed which demonstrated that s-CC was negatively correlated with the rate of decline of

Table 2 Multinomial logistic regression analysis between reverse dipper, non-dipper and dipper

Variable	Reverse dipper vs dipper		Non-dipper vs dipper		Reverse dipper vs non-dipper	
	OR (95% CI)	p Value	OR (95% CI)	p Value	OR (95% CI)	p Value
Diabetes mellitus	2.313 (1.401 to 3.821)	0.01	1.513 (0.969 to 2.361)	0.068	0.654 (0.437 to 0.977)	0.038
Triglycerides	0.704 (0.578 to 0.858)	<0.001	0.837 (0.74 to 0.95)	0.005	1.189 (0.986 to 1.434)	0.07
LDL-C	0.802 (0.63 to 1.022)	0.074	0.748 (0.609 to 0.919)	0.006	0.932 (0.755 to 1.152)	0.516
Cystatin C	1.717 (1.033 to 2.854)	0.037	1.493 (0.928 to 2.404)	0.010	0.87 (0.632 to 1.197)	0.391

LDL-C, low-density lipoprotein cholesterol.

nocturnal SBP ($r=-0.117$; $p=0.002$) and DBP ($r=-0.089$; $p=0.018$) (figure 1).

DISCUSSION

The present paper investigated the relationship between s-CC and circadian variations of BP. This is, to the best of our knowledge, the first study to report the association of s-CC level with BP reverse dipping. In addition, the decline rate of nocturnal SBP and DBP was negatively correlated with s-CC level, which was also confirmed using a linear regression model (unpublished results). Therefore, the association of BP reverse dipping with kidney damage implied that better control of night-time BP may protect the kidney.

The common circadian variation of BP is the physiological decline in nocturnal BP, more than 10% decrease compared with daytime BP, which is known as the dipper pattern of BP. Nocturnal BP is the minimal BP required for adequate organ perfusion in healthy

individuals.²⁰ However, the loss of the physiological decline in nocturnal BP is closely related to target organ damage and the renal damage is one of the most serious complications of hypertension. It has been reported that night-time BP bears a more significant predictive role for the risk of developing clinical events in hypertensive patients.^{21–24} Interestingly, reverse-dipper BP, with nocturnal BP higher than daytime BP, was found to be closely related to the progression and prognosis of renal and cardiovascular damage, increasing the risk of damages in heart, brain and kidney.^{11–25} The Ohasama study reported that the failure of nocturnal BP decline was significantly associated with a higher risk for cardiovascular mortality.²⁶ Consistently, our previous study also found that BP reverse dipping was the real risk factor for carotid atherosclerosis and lacuna infarction.¹⁶ In this study, we investigated the relationship between s-CC and BP patterns and found that s-CC level was increased significantly in the reverse-dipper group when compared with the dipper group.

Cystatin C, with a molecular weight of 13KD, is a cysteine protease inhibitor produced by nearly all human cells and filtered by the glomerulus.^{27–28} Compared to serum creatinine concentration s-CC is less affected by age, sex or muscle mass. Therefore, s-CC is considered to be a more sensitive marker of GFR, particularly for individuals with early kidney injury, which is prevalent in hypertensive patients, while those with poorly controlled BP are more prone to deteriorated GFR.^{2–3} In addition, there is emerging evidence indicating that possible associations may exist between s-CC and circadian BP rhythm, though the detailed mechanism remains to be further investigated. For example, Ordu *et al*¹⁵ found that serum levels of cystatin C were higher in the patients with non-dipper hypertension when compared with those in the dipper pattern. Surprisingly, different from other studies^{15–29} on the important prognostic value of non-dipper BP, our results revealed that s-CC (OR 1.717, CI 1.033 to 2.854, $p=0.037$) was significantly different between the reverse-dipper and dipper groups. By using cystatin C, we found a significant linear relationship between kidney function and the rate of decline of nocturnal BP in patients with essential hypertension.

Certain potential limitations should be considered. Owing to the limitation of the cross-sectional nature, long-term follow-up data were not provided and a longer period of prospective observation may provide more prognostic information. Second, we could not confirm the cause–effect relationship between reverse dipper BP and s-CC, or they may contribute to each other. In addition, this is a retrospective study, and the different living habits of participants do affect the accuracy of the results. Therefore, more prospective clinical observations or case–control studies are needed to investigate the role of this association between s-CC and reverse patterns of BP variability.

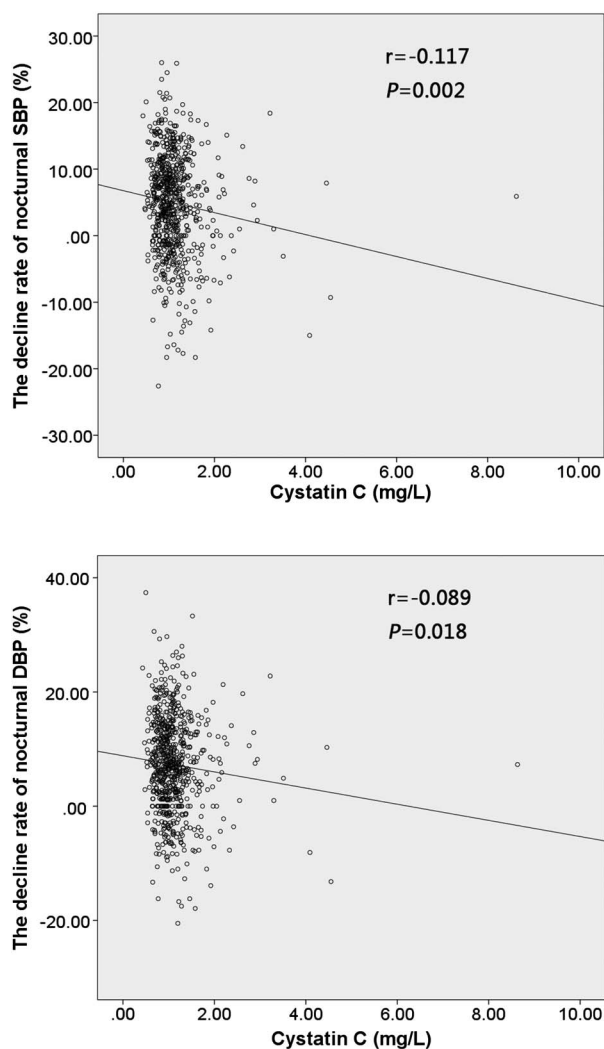


Figure 1 Correlation of cystatin C with the decline rate of nocturnal SBP and DBP. DBP, diastolic blood pressure; SBP, systolic blood pressure.

CONCLUSION

In conclusion, our results indicated that s-CC level was associated with the reverse-dipper pattern of BP examined with 24 hour ABPM.

Contributors JH, DS, KL and GW contributed to the design of the work. JH and DS collected the data. JH, YG, BY and YD wrote the manuscript. QG, YG, BY, LP and YD were involved in the analysis and interpretation of data. BY, KL and GW 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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