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A cross-sectional study on the relationship between the level of Serum cystatin C and blood pressure dipping status in hypertensive patients

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ABSTRACT

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

Design: Cross-sectional study.

Setting: Single center.

Participants: A total of 718 hypertensive patients were eventually included from general practices between 2012 and 2014 in the Second Affiliated Hospital, Xi'an Jiaotong University. Patients were excluded if they were<18 or >90 years old, under antihypertensive treatment, night workers, suffering acute stroke or myocardial infarction within the past 6 months, diagnosed as secondary hypertension or intolerant to the ABPM.

Measurement: The sample subjects were evaluated with 24 hours ambulatory BP monitoring (ABPM). Peripheral venous blood samples were collected to evaluate the s-CC levels by enzyme linked immune sorbent assay.

Methods: The distribution of hypertensive patients with different levels of s-CC among each circadian BP pattern group was analyzed using variance analysis. 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) was increased significantly when compared with dipper group (1.06±0.36)(P=0.021).In addition, after multinomial logistic regression analysis, s-CC (OR 1.717; 95% CI 1.033-2.854; P=0.037) and diabetes (OR 2.313; 95% CI 1.401-3.821; P=0.01) were significantly different between 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 s-CC level.

Conclusions: S-CC was associated with reverse-dipper pattern of BP examined with 24h ABPM. Moreover, the lower nocturnal decline rate of BP might increase the risk of early renal injury in hypertensive patients.

Strengths and limitations of this study

- The present study was the first to demonstrate that significantly elevated level of serum cystatin C (s-CC)in patients with reverse-dipper pattern of BP.
- Our study was a cross-sectional study, long-term follow-up data were not provided.
- The subjects in our study were recruited from a single center 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 approximately 50%.[1]Serum cystatin C（s-CC）is considered to be a more precise marker for compromised glomerular filtration rate 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 levels is important for clinical management of essential hypertensive 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 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 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 blood pressures 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 is higher in patients with "non-dipper" hypertension patients 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 center, 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 ABPM service database in our hospital. Patients were excluded if they were<18 or >90 years old, under antihypertensive treatment, night workers, suffering acute stroke or myocardial infarction within the past 6 months, diagnosed as secondary hypertension or intolerant to the ABPM. The study protocol was approved by the Ethics Committee of the Second Affiliated Hospital, Xi'an Jiaotong University. All the patients were referred due to standard indications that have been shown to use ABPM for appropriate clinical circumstance.

We considered subjects to have clinical hypertension if their systolic BP(SBP)>140mm Hg and/or diastolic BP (DBP)>90 mm Hg in casual office recording, or if their 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.[18] Decline rate of nocturnal SBP was calculated as (daytime SBP–nighttime SBP)*100/daytime BP. 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 (10%–20% SBP fall), non-dipper (0%–10% SBP fall), and reverse dipper (<0% SBP fall), according to the range of the nocturnal SBP dip.[13]

Measurement

All the hypertensive patients were subject to24-hour ABPM using an oscillometric device (Spacelabs 90207; Spacelabs, Redmond, WA). The arm cuff was fixed to the non-dominant upper limb between 7:00 and $9:00_{AM}$ and removed 24 hours later. BP was recorded every 15 minutes from $7:00_{AM}$ to $11:00_{PM}$ (daytime BP values) and every 30 minutes from $11:00_{PM}$ to $7:00_{AM}$ (nocturnal BP values).For each24-hour ABPM, daytime BP and nighttime 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 manually edited by 2 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 beats per-minute.

Serum samples were obtained in the morning after a fasting period of 12h. 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±SD for continuous normally distributed variables. To compare ordinal and continuous normally distributed variables between subgroups of circadian BP and s-CC, chi-squared and analysis of variance (ANOVA) were employed, respectively. 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. Variables were included in the multivariate analyses, which were found significantly in univariate models. The relationship between two continuous variables was assessed by bivariate correlation analysis (Pearson's correlation). Multinomial logistic regression was also employed to analyze the relationship between circadian BP (dipper, non-dipper and reverse dipper) and clinical variable. A calculated difference of $P<0.05$ was considered to be statistically significant. All the data was analyzed using SPSS 18.0 (SPSS Inc).

RESULTS

Baseline characteristics

The clinical characteristics of study population in different groups according to dipping status were shown in Table 1, Among 718 participants, the average age was 59.6±13.8years and 54% were male. The mean s-CC level was 1.1±0.5 mg/L. Mean 24-h SBP of 24 hours was 135.6±14.2 mm Hg and mean DBP 79.6±10.5 mm Hg. In our study, reverse-dipper BP pattern was observed in 171 patients (23.8%) and dipper pattern in 177 patients (24.7%).A total of 370 hypertensive patients (51.5%) had non-dipper pattern. Compared with dipper BP pattern, subjects of 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). S-CC level in reverse-dipper group

(1.19±0.53) was increased significantly 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.

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#*	＜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 h-SBP, ABPM, mm Hg	134.16±13.28	135.44±13.79	137.23±15.72#	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#	142.52±16.84#*	＜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#*	0.004
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

Explanatory footnote: ABPM, ambulatory blood pressure monitoring; DBP, diastolic blood pressure; SBP: systolic blood pressure; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; VLD-C: very low density lipoprotein cholesterol.

#Indicated control with dipper group P<0.05.

*Indicated control with non-dipper group P<0.05.

Association of s-CC with reverse dipper pattern

In order to evaluate the association of different circadian BP patterns with s-CC level, multinomial regression analyses were performed. The variables included diabetes, triglycerides, low-density lipoprotein

cholesterol(LDL-C),s-CC found significantly and accepted collinearity in univariate models was included in the multinomial analyses. It was discovered that diabetes (OR 2.313,95%CI 1.401~3.821; *P*=0.01), triglycerides (OR 0.704, 95%CI 0.578~0.858;*P*<0.001), and s-CC(OR 1.717,95%CI 1.033~2.854;*P*=0.037) were significantly different between reverse dipper and dipper (Table 2).Moreover, triglycerides（OR 0.837；95% CI 0.74~0.948；*P*= 0.005）and LDL-C（OR 0.75；95%CI 0.61~0.92；*P*= 0.006）were significantly increased in non-dipper compared with dipper BP pattern. However, s-CC、triglycerides and LDL-C was not significantly different between non-dipper and dipper (Table 2).

Table 2. Multinomial logistic regression analysis between Reverse Dipper, Non-dipper, and Dipper

	Reverse Dipper vs Dipper		Nondippervs Dipper		Reverse Dipper vsNondipper	
Variable	OR(95% CI)	*P*	OR(95% CI)	*P*	OR(95% CI)	*P*
Diabetes mellitus	2.313(1.401~3.821)	0.01	1.513(0.969~2.361)	0.068	0.654(0.437~0.977)	0.038
Triglycerides	0.704(0.578~0.858)	＜0.001	0.837(0.74~0.95)	0.005	1.189(0.986~1.434)	0.07
LDL-C	0.802(0.63~1.022)	0.074	0.748(0.609~0.919)	0.006	0.932(0.755~1.152)	0.516
Cystatin C	1.717(1.033~2.854)	0.037	1.493(0.928~2.404)	0.0099	0.87(0.632~1.197)	0.391

CI=confidence interval, OR=odds ratio, LDL-C, low-density lipoprotein cholesterol

Correlation between s-CC Level and Decline Rate of Nocturnal BP

Nocturnal BP is strongly associated with hypertensive target organ damage. We assessed the effect of the nocturnal decline of BP on prognosis by using the decline rate of nocturnal BP as a continuous variable, which also shows the BP dipping status.[20] The decline rate of nocturnal BP the extent of the decline in nocturnal BP was related to the cardiovascular mortality.[21] In order to further investigate the relationship between different BP patterns and s-CC, bivariate correlation analysis was performed. Consistently, we found that s-CC was negatively correlated with the rate of decline nocturnal SBP (r=-0.117; *p*=0.002) and DBP (r=-0.089; *p*=0.018) (Figure 1).

Discussion

The common circadian variation of BP is the physiologic decline in nocturnal BP, more than 10% decrease compared with daytime BP, which is known as dipper pattern of BP. Nocturnal BP is the minimal BP required for adequate organ perfusion in healthy individuals.[22] However, the loss of the physiologic 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 nighttime BP bear a more significant predictive role for the risk of developing clinical events in hypertensive patients.[23-26] 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 organ damages in heart, brain and kidney.[27, 28]The Ohasama study reported that the failure of nocturnal BP decline was significantly associated with a higher risk for cardiovascular mortality.[21] Consistently, our previous study also found that BP reverse dipping was the real risk factor for carotid atherosclerosis and lacuna infarction.

Cystatin C, a molecular weight of 13KD, is a cysteine protease inhibitor produced by nearly all human cells and filtered by the glomerulus.[29, 30] 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.[2, 3]Additionally, there were a lot of studies showed that s-CC was involved in the pathological and physiological process of cardiovascular disease. Prats *et al*.[31] provided evidence that s-CC is independently related to left ventricular mass and could be a marker for hypertensive cardiac hypertrophy. Muntner *et al*.[32] demonstrated an association between s-CC and cardiovascular disease prevalence in patients without chronic kidney disease. Watanabe S *et al*. [4] has found the relationship between s-CC level and end-organ

damages in the kidney, heart, and vessels of patients with essential hypertension, and s-CC was closely associated with essential hypertension. C. Mena *et al*. [33]reported that a association exists between s-CC and both SBP and pulse pressure, using hospital blood pressure measurement with a classic mercury sphygmomanometer, in cohort of patients throughout a wide range of kidney function levels. These findings may greatly enhance our discovery that s-CC is strongly associated with circadian BP variations.

Early kidney injury is prevalent in hypertensive patients, while those with poorly controlled blood pressure are more prone to deteriorated glomerular filtration rate. Moreover, there is an emerging evidence indicating that the possible associations may exist between s-CC and circadian BP rhythm, though the detailed mechanism remains to be further investigated. For example, Serkan*et al*. [15] found that serum levels of cystatin C was higher in the patients with non-dipper hypertension when compared to those in the dipper pattern. Surprisingly, different from other studies on the important prognostic value of non-dipper, our results revealed that s-CC (OR 1.717,CI 1.033~2.854,P=0.037)were significantly different between reverse-dipper and dipper. 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. However, several studies have reported that s-CC was involved in the pathological and physiological process of cardiovascular disease, the pathophysiological mechanism is incompletely understood. Therefore, we cannot get the cause-effect relationship between cystatin C and BP pattern.

For the first time, we evaluated s-CC level in patients with reverse-dipper pattern of BP and found that s-CC level was increased significantly in reverse-dipper group when compared with dipper group. Moreover, the lower nocturnal decline rate of BP might increase the risk of s-CC and early renal injury in hypertensive patients probably. However, due 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. Secondly, we cannot confirm any direct relationship between reverse dipper and s-CC, or they may contribute to each other. Therefore, more prospective clinical observation is needed to investigate the role and the mechanism of this association between s-CC and reverse patterns of BP variability.

Conclusion

In conclusion, our results confirmed that s-CC was associated with reverse-dipper pattern of BP examined with 24h ABPM and that s-CC level was increased significantly in reverse-dipper group when compared with dipper group.

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. GW, BY, KL and YD 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.

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Data sharing statement No additional data are available.

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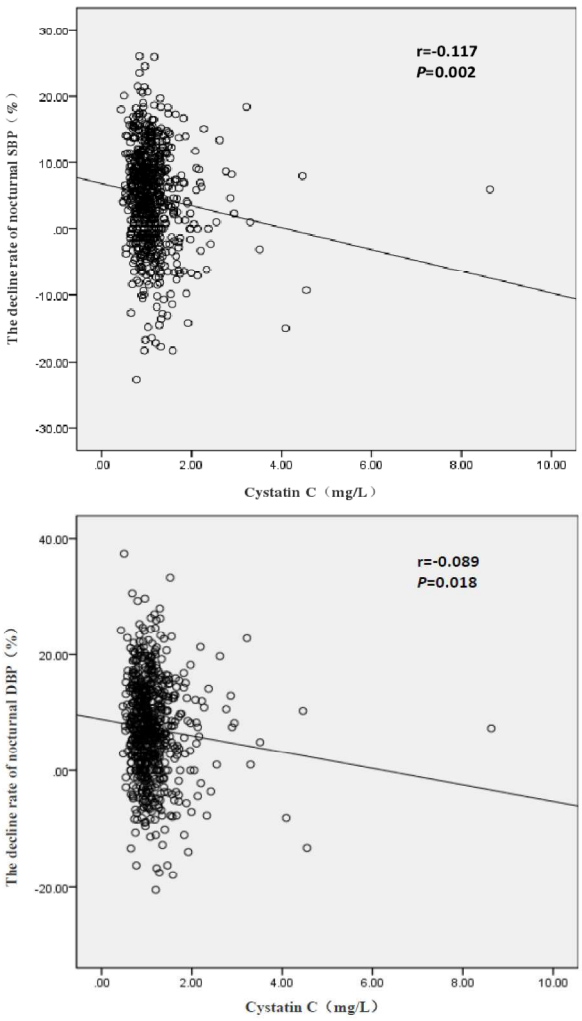

Figure 1. Correlation of cystatin C with the decline rate of nocturnal SBP and DBP.
DBP, diastolic blood pressure; SBP, systolic blood pressure.

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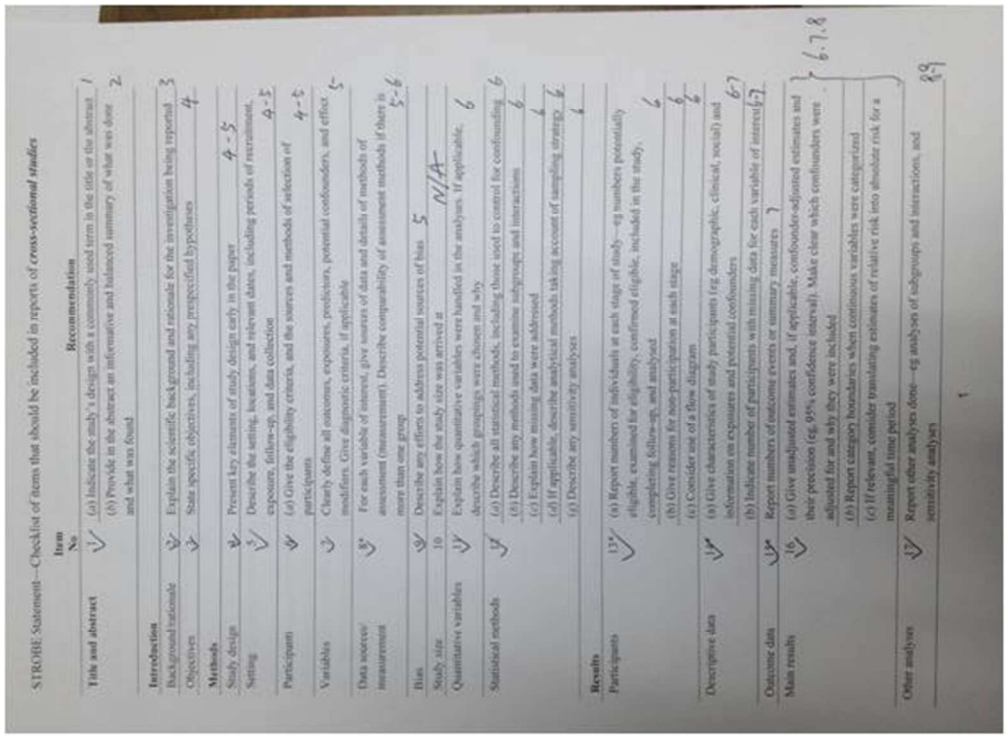

STROBE Statement—Checklist of items that should be included in reports of *cross-sectional studies*

	Item No	Recommendation	
Title and abstract	√	(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	√	Explain the scientific background and rationale for the investigation being reported	3
Objectives	√	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	√	Present key elements of study design early in the paper	4-5
Setting	√	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5
Participants	√	(a) Give the eligibility criteria, and the sources and methods of selection of participants	4-5
Variables	√	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/ measurement	√	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	5-6
Bias	√	Describe any efforts to address potential sources of bias	5
Study size	√	Explain how the study size was arrived at	N/A
Quantitative variables	√	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	√	(a) Describe all statistical methods, including those used to control for confounding	6
		(b) Describe any methods used to examine subgroups and interactions	6
		(c) Explain how missing data were addressed	6
		(d) If applicable, describe analytical methods taking account of sampling strategy	6
		(e) Describe any sensitivity analyses	6
Results			
Participants	√	(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	8
		(c) Consider use of a flow diagram	8
Descriptive data	√	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6-7
		(b) Indicate number of participants with missing data for each variable of interest	6-7
Outcome data	√	Report numbers of outcome events or summary measures	7
Main results	√	(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	6, 7, 8
		(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	√	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8,9

103x75mm (150 x 150 DPI)

Discussion			
Key results	✓	Summarise key results with reference to study objectives	9-10
Limitations	✓	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	✓	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9-10
Generalisability	✓	Discuss the generalisability (external validity) of the study results	11
Other information			
Funding	✓	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	11

*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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A 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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A 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 serum cystatin C (s-CC) and reverse-dipper blood pressure (BP) pattern.

Design: Cross-sectional study.

Setting: Single center.

Participants: A total of 718 hypertensive patients were eventually included from general practices between 2012 and 2014 in the Second Affiliated Hospital, Xi'an Jiaotong University. Participants were recruited from cardiac clinics if they have clinical hypertension when their systolic BP (SBP)>140mm Hg and/or diastolic BP (DBP)>90 mm Hg in casual office recording. Patients were excluded if they were<18 or >90 years old, under antihypertensive treatment, night workers, suffering acute stroke or myocardial infarction within the past 6 months, diagnosed as secondary hypertension, sleep apnoea or other sleep disorders, renal failure, cardiac failure, COPD or women during pregnancy or intolerant to the ABPM.

Measurement: The sample subjects were evaluated with 24 hours ambulatory BP monitoring (ABPM). Peripheral venous blood samples were collected to evaluate the s-CC levels by enzyme linked immune sorbent assay.

Methods: The distribution of hypertensive patients with different levels of s-CC among each circadian BP pattern group was analyzed using analysis of variance (ANOVA). 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 1.033-2.854; P=0.037) and diabetes (OR 2.313; 95% CI 1.401-3.821; P=0.01) were significantly different between 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 s-CC level.

Conclusions: S-CC was associated with reverse-dipper pattern of BP examined with 24h ABPM. Moreover, the lower nocturnal decline rate of BP might increase the risk of early renal injury in hypertensive patients.

Strengths and limitations of this study

- The present study was the first to demonstrate that significantly elevated level of serum cystatin C (s-CC) in patients with reverse-dipper pattern of BP.
- Our study was a cross-sectional study, long-term follow-up data were not provided.
- The subjects in our study were recruited from a single center 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 approximately 50%.[1] Serum cystatin C（s-CC）is considered to be a more precise marker for compromised glomerular filtration rate 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 levels is important for clinical management of essential hypertensive 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 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 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 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 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 blood pressures 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 is higher in patients with "non-dipper" hypertension patients 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 center, 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 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 apnea syndrome, secondary hypertension, could not tolerate the ABPM, pregnant female, sleep apnea syndrome, arrhythmia, congestive heart failure, hepatic failure, kidney failure and chronic obstructive pulmonary Disease (COPD). The

study protocol was approved by the Ethics Committee of the Second Affiliated Hospital, Xi'an Jiaotong University. All the patients were referred due to standard indications that have been shown to use ABPM for appropriate clinical circumstance.

We considered subjects to have clinical hypertension if their systolic BP(SBP)>140mm Hg and/or diastolic BP (DBP)>90 mm Hg in casual office recording, or if their 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.[18] Decline rate of nocturnal SBP was calculated as (daytime SBP–nighttime SBP)*100/daytime BP. 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 (>= 10% and < 20% SBP fall), non-dipper (>= 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 subject to24-hour ABPM using an oscillometric device (Spacelabs 90207; Spacelabs, Redmond, WA). The arm cuff was fixed to the non-dominant upper limb between 7:00 and $9:00_{AM}$ removed 24 hours later. BP was recorded every 15 minutes from $7:00_{AM}$ to $11:00_{PM}$ (daytime BP values) and 30 minutes from $11:00_{PM}$ to $7:00_{AM}$ (nocturnal BP values). For each24-hour ABPM, daytime BP and nighttime 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 manually edited by 2 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, heart rate<40 or >150 beats/minute. Serum samples were obtained in the morning after a fasting period of 12h.

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 ± SD for continuous normally distributed variables. To compare ordinal and continuous normally distributed variables between subgroups of circadian BP and s-CC, chi-squared and analysis of variance (ANOVA) were employed, respectively. Correlation between s-CC level, as well as the decline rate of nocturnal SBP and DBP was assessed by bivariate correlation analysis (Pearson's correlation). Multinomial logistic regression was also employed to analyze the relationship between circadian BP (dipper, non-dipper and reverse dipper) and the clinical relevant variables. A calculated difference of $P<0.05$ was considered to be statistically significant. All the data was analyzed using SPSS 18.0 (SPSS Inc).

RESULTS

Baseline characteristics

The clinical characteristics of study population in different groups according to dipping status were shown in 1, Among 718 participants, the average age was 59.6±13.8 years and 54% were male. The mean s-CC level was 1.1±0.5 mg/L. Mean 24-h SBP of 24 hours was 135.6±14.2 mm Hg and mean DBP 79.6±10.5 mm Hg. In our reverse-dipper BP pattern was observed in 171 patients (23.8%) and dipper pattern in 177 patients (24.7%). A of 370 hypertensive patients (51.5%) had non-dipper pattern. Compared with dipper BP pattern, subjects of 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). S-CC level in reverse-dipper group (1.19±0.53) was

significantly 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.

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(28.24%)	53(14.32%)	9(5.26%)	＜0.001
SH, n (84.40%)	127(71.75%)	317(85.68)	162(94.73%)	＜0.001
Age, y	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 h- 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 h-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

Explanatory footnote: ABPM, ambulatory blood pressure monitoring; DBP, diastolic blood pressure; SBP: systolic blood pressure; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; VLD-C: very low density lipoprotein cholesterol; WCH: white coat hypertension (office BP ⩾140/90 mm Hg with normal ABPM measurements) ; MH; masked hypertension(office BP < 140/80 with high ABPM measurements).

#Indicated control with dipper group P<0.05.

*Indicated control with non-dipper group P<0.05.

Association of s-CC with reverse dipper 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 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 univariate models, multinomial regression analyses were carried out and indicated that S-CC (OR 1.717, 95%CI 1.033~2.854; *P*=0.037) were significantly different between BP reverse dipping and dipping groups (Table 2). It was also discovered that diabetes (OR 2.313,95%CI 1.401~3.821; *P*=0.01), triglycerides (OR 0.704, 95%CI 0.578~0.858;*P*<0.001) were significantly different between these two groups (Table 2).

Table 2. Multinomial logistic regression analysis between Reverse Dipper, Non-dipper, 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*
Diabetes mellitus	2.313(1.401~3.821)	0.01	1.513(0.969~2.361)	0.068	0.654(0.437~0.977)	0.038
Triglycerides	0.704(0.578~0.858)	＜0.001	0.837(0.74~0.95)	0.005	1.189(0.986~1.434)	0.07
LDL-C	0.802(0.63~1.022)	0.074	0.748(0.609~0.919)	0.006	0.932(0.755~1.152)	0.516
Cystatin C	1.717(1.033~2.854)	0.037	1.493(0.928~2.404)	0.0099	0.87(0.632~1.197)	0.391

CI=confidence interval, OR=odds ratio, LDL-C, low-density lipoprotein cholesterol

Correlation between s-CC Level and 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 and demonstrated that s-CC was negatively correlated with the rate of decline nocturnal SBP (r=-0.117; *p*=0.002) and DBP (r=-0.089; *p*=0.018) (Figure 1).

Discussion

The common circadian variation of BP is the physiologic decline in nocturnal BP, more than 10% decrease compared with daytime BP, which is known as dipper pattern of BP. Nocturnal BP is the minimal BP required for adequate organ perfusion in healthy individuals.[20] However, the loss of the physiologic 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 nighttime BP bear 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 organ damages in heart, brain and kidney.[25, 26] The Ohasama study reported that the failure of nocturnal BP decline was significantly associated with a higher risk for cardiovascular mortality.[27] Consistently, our previous study also found that BP reverse dipping was the real risk factor for carotid atherosclerosis and lacuna infarction. In this study, we investigate the relationship between s-CC and BP patterns and found that s-CC level was increased significantly in reverse-dipper group when compared with dipper group.

Cystatin C, a molecular weight of 13KD, is a cysteine protease inhibitor produced by nearly all human cells and filtered by the glomerulus.[28, 29] 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 blood pressure are more prone to deteriorated glomerular filtration rate[2, 3]. In addition, there is an emerging evidence indicating that the possible associations may exist between s-CC and circadian BP rhythm, though the detailed mechanism remains to be further investigated. For example, Serkan*et al.* [15] found that serum levels of cystatin C

was higher in the patients with non-dipper hypertension when compared to those in the dipper pattern. Surprisingly, different from other studies[15, 30] on the important prognostic value of non-dipper, our results revealed that s-CC (OR 1.717,CI 1.033~2.854,P=0.037) were significantly different between reverse-dipper and dipper. 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. However, several studies have reported that s-CC was also involved in the pathological and physiological process of cardiovascular disease, the pathophysiological mechanism is incompletely understood.

For the first time, we evaluated s-CC level in patients with reverse-dipper pattern of BP and found that s-CC level was increased significantly in reverse-dipper group when compared with dipper group. Moreover, the lower nocturnal decline rate of BP might increase the risk of s-CC and early renal injury in hypertensive patients probably. The clinical relevance of the association of reverse dipper hypertension with kidney damage we report could attach more attention to the management of reverse dipper hypertension, because the better control of nighttime blood pressure may protect the kidney in clinics. However, due 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. Secondly, we cannot confirm cause-effect relationship between reverse dipper and s-CC, or they may contribute to each other. In addition, this is a retrospective study, and the different living habit of participants does affect the accuracy of the results. Therefore, more prospective clinical observations or case-control studies are needed to investigate the role and the mechanism of this association between s-CC and reverse patterns of BP variability.

Conclusion

In conclusion, our results confirmed that s-CC was associated with reverse-dipper pattern of BP examined with 24h ABPM and that s-CC level was increased significantly in reverse-dipper group when compared with dipper group.

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.

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Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

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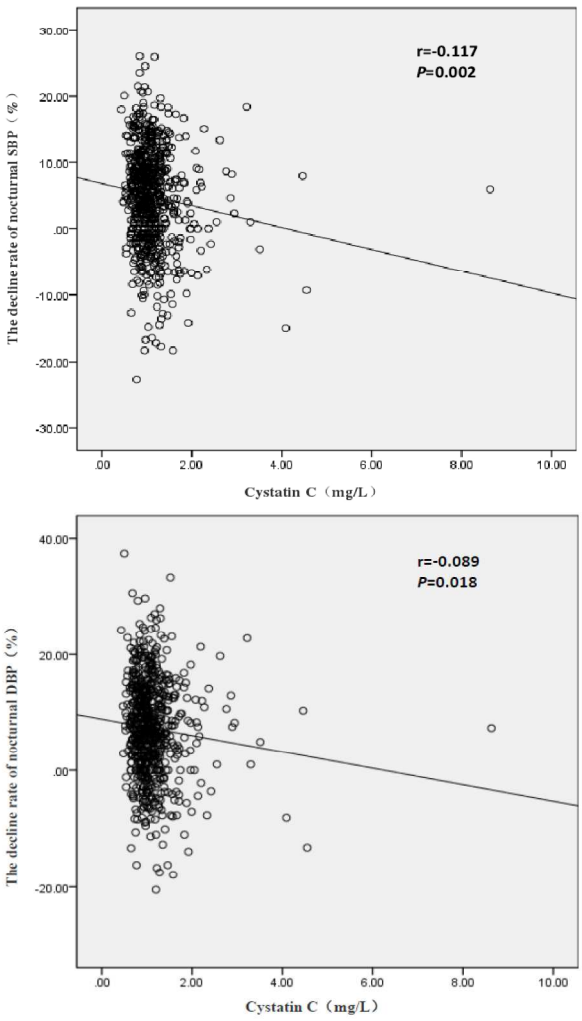

Figure 1. Correlation of cystatin C with the decline rate of nocturnal SBP and DBP.
DBP, diastolic blood pressure; SBP, systolic blood pressure.

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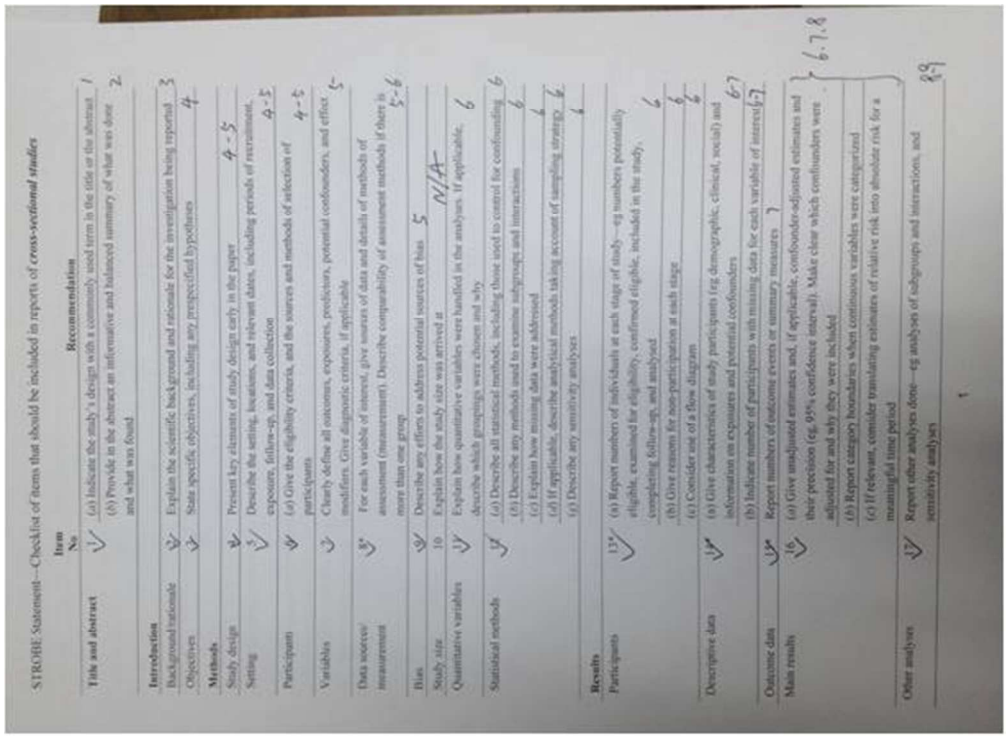

STROBE Statement—Checklist of items that should be included in reports of *cross-sectional studies*

	Item No	Recommendation	
Title and abstract	√	(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	√	Explain the scientific background and rationale for the investigation being reported	3
Objectives	√	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	√	Present key elements of study design early in the paper	4-5
Setting	√	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5
Participants	√	(a) Give the eligibility criteria, and the sources and methods of selection of participants	4-5
Variables	√	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/ measurement	√	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	5-6
Bias	√	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	√	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	√	(a) Describe all statistical methods, including those used to control for confounding	6
		(b) Describe any methods used to examine subgroups and interactions	6
		(c) Explain how missing data were addressed	6
		(d) If applicable, describe analytical methods taking account of sampling strategy	6
		(e) Describe any sensitivity analyses	6
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	6
		(b) Give reasons for non-participation at each stage	6
		(c) Consider use of a flow diagram	6
Descriptive data	14* √	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6-7
		(b) Indicate number of participants with missing data for each variable of interest	6-7
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	6, 7, 8
		(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	√	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8,9

103x75mm (150 x 150 DPI)

Discussion			
Key results	✓	Summarise key results with reference to study objectives	9-10
Limitations	✓	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	✓	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9-10
Generalisability	✓	Discuss the generalisability (external validity) of the study results	11
Other information			
Funding	✓	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	11

*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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A 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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Primary Subject Heading:	Renal medicine
Secondary Subject Heading:	Cardiovascular medicine
Keywords:	blood pressure, essential hypertension, reverse dipping, serum cystatin C

A 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 serum cystatin C (s-CC) and reverse-dipper blood pressure (BP) pattern.

Design: Cross-sectional study.

Setting: Single center.

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 BP (SBP) and/or diastolic BP (DBP). Patients were excluded if they were<18 or >90 years old, under antihypertensive treatment, night workers, suffering acute stroke or myocardial infarction within the past 6 months, diagnosed as secondary hypertension, sleep apnoea or other sleep disorders, renal failure, cardiac failure, COPD or women during pregnancy or intolerant to the ABPM.

Measurement: The sample subjects were evaluated with 24 hours ambulatory BP monitoring (ABPM). Peripheral venous blood samples were collected to evaluate the s-CC levels by enzyme linked immune sorbent assay.

Methods: The distribution of hypertensive patients with different levels of s-CC among each circadian BP pattern group was analyzed using analysis of variance (ANOVA). 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 1.033-2.854; P=0.037) and diabetes (OR 2.313; 95% CI 1.401-3.821; P=0.01) were significantly different between 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 s-CC level.

Conclusions: S-CC level was increased significantly in reverse-dipper group when compared with dipper group and that s-CC was associated with reverse-dipper pattern of BP examined with 24h ABPM.

Strengths and limitations of this study

- The present study was the first to demonstrate that significantly elevated level of serum cystatin C (s-CC) in patients with reverse-dipper pattern of BP.
- Our study was a cross-sectional study, long-term follow-up data were not provided.
- The subjects in our study were recruited from a single center 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 approximately 50%.[1] Serum cystatin C（s-CC）is considered to be a more precise marker for compromised glomerular filtration rate 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 clinical management of essential hypertensive 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 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 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 blood pressures 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 center, 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 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 apnea syndrome, secondary hypertension, could not tolerate the ABPM, pregnant female, sleep apnea syndrome, arrhythmia,

congestive heart failure, hepatic failure, kidney failure and chronic obstructive pulmonary disease (COPD). The study protocol was approved by the Ethics Committee of the Second Affiliated Hospital, Xi'an Jiaotong University. All the patients were referred to standard indications that have been shown to use ABPM for appropriate clinical circumstance.

We considered subjects to have clinical hypertension if their systolic BP(SBP)>140mm Hg and/or diastolic BP (DBP)>90 mm Hg in casual office recording, or if their 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.[18] Decline rate of nocturnal BP was calculated as (daytime BP–nighttime BP)*100/daytime BP. 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 (>= 10% and < 20% SBP fall), non-dipper (>= 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 subject to 24-hour ABPM using an oscillometric device (Spacelabs 90207; Spacelabs, Redmond, WA). The arm cuff was fixed to the non-dominant upper limb between 7:00 and $9:00_{AM}$ and removed 24 hours later. BP was recorded every 15 minutes from $7:00_{AM}$ to $11:00_{PM}$ (daytime BP values) and every 30 minutes from $11:00_{PM}$ to $7:00_{AM}$ (nocturnal BP values). For each 24-hour ABPM, daytime BP and nighttime 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 2 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 beats/minute. Serum samples were obtained in the morning after a fasting period of 12h. 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 ± SD for continuous normally distributed variables. To compare ordinal and continuous normally distributed variables between subgroups of circadian BP and s-CC, chi-squared 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 analyze the relationships between circadian BP patterns (dipper, non-dipper and reverse dipper) and related risk factors. Any variable with *P* values less than 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 blood pressure level and s-CC level. A calculated difference of $P<0.05$ was considered to be statistically significant. All the data was analyzed using SPSS 18.0 (SPSS Inc).

RESULTS

Baseline Characteristics

The clinical characteristics of study population in different groups according to dipping status were shown in Table 1, Among 718 participants, the average age was 59.6±13.8 years and 54% were male. The mean s-CC level was 1.1±0.5 mg/L. Mean 24-h SBP of 24 hours was 135.6±14.2 mm Hg and mean DBP 79.6±10.5 mm Hg.

In our study, reverse-dipper BP pattern was observed in 171 patients (23.8%) and dipper pattern in 177 patients (24.7%). A total of 370 hypertensive patients (51.5%) had non-dipper pattern. Compared with dipper BP pattern, subjects of 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). S-CC level in reverse-dipper group (1.19±0.53) was increased significantly 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.

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, y	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 h- 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 h-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

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

measurements).

#Indicated control with dipper group $P<0.05$.

*Indicated control with non-dipper group $P<0.05$.

Association of S-CC with Reverse Dipper 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 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 univariate models, multinomial regression analyses were carried out and indicated that s-CC (OR 1.717, 95%CI 1.033~2.854; P=0.037) was significantly different between BP reverse dipping and dipping groups (Table 2). It was also discovered that diabetes (OR 2.313, 95%CI 1.401~3.821; P=0.01), triglycerides (OR 0.704, 95%CI 0.578~0.858; $P<0.001$) were significantly different between these two groups (Table 2).

Table 2. Multinomial logistic regression analysis between Reverse Dipper, Non-dipper 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*
Diabetes mellitus	2.313(1.401~3.821)	0.01	1.513(0.969~2.361)	0.068	0.654(0.437~0.977)	0.038
Triglycerides	0.704(0.578~0.858)	＜0.001	0.837(0.74~0.95)	0.005	1.189(0.986~1.434)	0.07
LDL-C	0.802(0.63~1.022)	0.074	0.748(0.609~0.919)	0.006	0.932(0.755~1.152)	0.516
Cystatin C	1.717(1.033~2.854)	0.037	1.493(0.928~2.404)	0.010	0.87(0.632~1.197)	0.391

CI=confidence interval, OR=odds ratio, LDL-C, low-density lipoprotein cholesterol

Correlation between S-CC Level and 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 and demonstrated that s-CC was negatively correlated with the rate of decline 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 models (unpublished results). Therefore, the association of BP reverse dipping with kidney damage implied that the better control of nighttime blood pressure may protect the kidney in clinics.

The common circadian variation of BP is the physiologic decline in nocturnal BP, more than 10% decrease compared with daytime BP, which is known as dipper pattern of BP. Nocturnal BP is the minimal BP required for adequate organ perfusion in healthy individuals.[20] However, the loss of the physiologic 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 nighttime BP bear 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 organ 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 reverse-dipper group when compared with dipper group.

Cystatin C, 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 blood pressure are more prone to deteriorated glomerular filtration rate[2, 3]. In addition, there is emerging evidence indicating that the possible associations may exist between s-CC and circadian BP rhythm, though the detailed mechanism remains to be further investigated. For example, Serkan*et al.* [15] found that serum levels of cystatin C were higher in the patients with non-dipper hypertension when compared to those in the dipper pattern. Surprisingly, different from other studies[15, 29] on the important prognostic value of non-dipper, our results revealed that s-CC (OR 1.717, CI 1.033~2.854, *P*=0.037) was significantly different between reverse-dipper and dipper. 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. Due 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. Secondly, we couldn't confirm cause-effect relationship between reverse dipper and s-CC, or they may contribute to each other. In addition, this is a retrospective study, and the different living habit of participants does 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.

Conclusion

In conclusion, our results confirmed that s-CC level was increased significantly in reverse-dipper group when compared with dipper group and that s-CC was associated with reverse-dipper pattern of BP examined with 24h 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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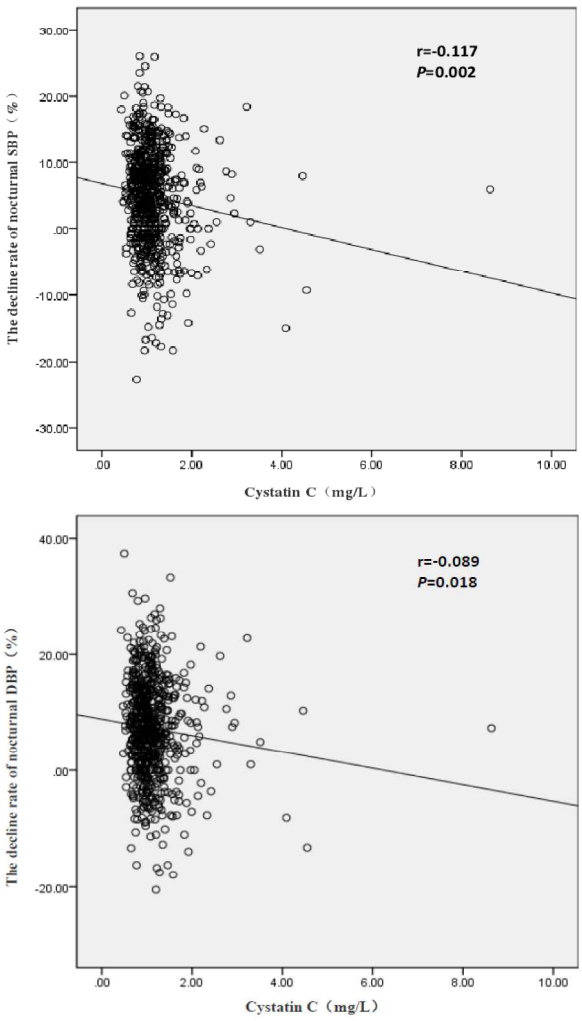

Figure 1. Correlation of cystatin C with the decline rate of nocturnal SBP and DBP.
DBP, diastolic blood pressure; SBP, systolic blood pressure.

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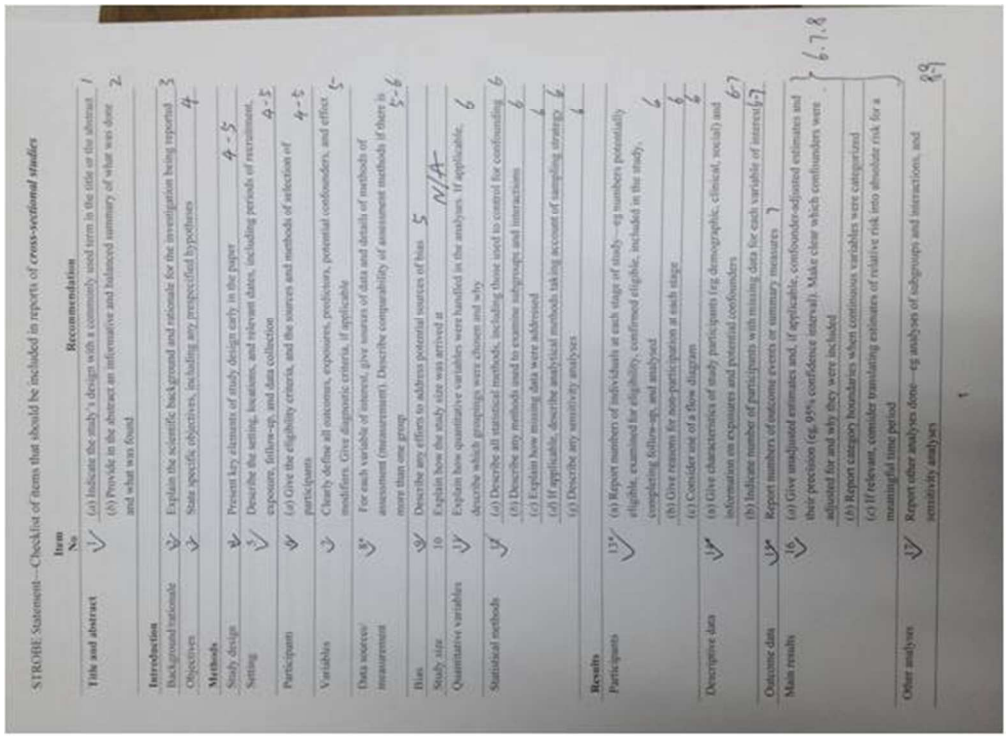

STROBE Statement—Checklist of items that should be included in reports of *cross-sectional studies*

	Item No	Recommendation	
Title and abstract	√	(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	√	Explain the scientific background and rationale for the investigation being reported	3
Objectives	√	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	√	Present key elements of study design early in the paper	4-5
Setting	√	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5
Participants	√	(a) Give the eligibility criteria, and the sources and methods of selection of participants	4-5
Variables	√	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/ measurement	√	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	5-6
Bias	√	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	√	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	√	(a) Describe all statistical methods, including those used to control for confounding	6
		(b) Describe any methods used to examine subgroups and interactions	6
		(c) Explain how missing data were addressed	6
		(d) If applicable, describe analytical methods taking account of sampling strategy	6
		(e) Describe any sensitivity analyses	6
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	6
		(b) Give reasons for non-participation at each stage	6
		(c) Consider use of a flow diagram	6
Descriptive data	14* √	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6-7
		(b) Indicate number of participants with missing data for each variable of interest	6-7
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	6, 7, 8
		(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	√	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8,9

103x75mm (150 x 150 DPI)

Discussion			
Key results	✓	Summarise key results with reference to study objectives	9-10
Limitations	✓	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	✓	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9-10
Generalisability	✓	Discuss the generalisability (external validity) of the study results	11
Other information			
Funding	✓	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	11

*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.

2

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