Int J Clin Exp Med 2016;9(11):22012-22019
www.ijcem.com /ISSN:1940-5901/IJCEM0036848

Original Article

Relationship of serum homocysteine level with the occurrence of aneurysmal subarachnoid hemorrhage and poor outcome

Xiu Liu[1*], Xiaochu Chen[2*], Yang Liu[1*], Xuyi Chen[3], Ya Su[4*], Hao Fu[3], Haiyang Yan[3], Shengkai Sun[2], Zhihong Wang[1]

[1]Postgraduate Training Base, Affiliated Hospital of Logistics University of Chinese People's Armed Police Force (CAPF), Jinzhou Medical University, No. 220, Chenglin Road, Hedong District, Tianjin 300162, China; [2]Department of Medical Education of CAPF, No. 220, Chenglin Road, Hedong District, Tianjin 300162, China; [3]Neurology and Neurosurgery Hospital of CAPF, Tianjin Key Laboratory of Neuro-trauma Repair, Tianjin, China; [4]Neurology and Neurosurgery Hospital of CAPF, Tianjin, China. [*]Co-first authors.

Received July 29, 2016; Accepted August 30, 2016; Epub November 15, 2016; Published November 30, 2016

Abstract: Although serum homocysteine (Hcy) is closely correlated with the incidence of cerebrovascular diseases such as progressive stroke and aneurysmal subarachnoid hemorrhage (aSAH), the relationship of laboratory indices, especially serum Hcy at hospital admission with the occurrence and prognosis of aSAH has not been revealed. To investigate the relationship of admission serum Hcy level with the occurrence of aSAH and poor hospital discharge outcome. In this retrospective case-control study, patients with aSAH (aSAH group; n = 125) and healthy subjects (control group; n = 125) were enrolled. Clinical data including patient demographics, physical examination, imaging data, and laboratory investigations were collected. Unconditional logistic regression analysis was employed to reveal the relationship of admission serum Hcy level and other laboratory indices with the occurrence of aSAH. Poor hospital discharge outcome was defined as the Glasgow Outcome Scale score < 4 points. All the patients in the aSAH group were stratified into four groups using quartile method based on serum Hcy level and comparison was made between the Hcy levels of poor and good outcome groups. In multivariate logistic regression analysis, serum Hcy, blood glucose, uric acid, systolic blood pressure, and diastolic blood pressure were found to be significantly different between aSAH and control groups. In relative risk analysis, the first, third, and fourth quartile groups had 2.444-, 3.500-, and 4.978-fold risks for poor outcome compared with the second quartile group (optimal outcome group). Admission serum Hcy is independent risk factor for aSAH and is correlated with poor hospital discharge outcome.

Keywords: Aneurysmal subarachnoid hemorrhage (aSAH), aneurysm, serum homocysteine, prognosis, risk factor

Introduction

Aneurysmal subarachnoid hemorrhage (aSAH) is a common cerebrovascular disease (CVD) characterized by acute onset and poor outcome [1]. In the recent epidemiological survey, the reported median mortality rate in the United States of America was 32% while it was 43%~44% in Europe. With the overall rise in morbidity rate [2], it poses a huge health and economic thread to the public [3].

Homocysteine (Hcy), a highly active sulfur-containing amino acid, is synthesized through enzymatic hydrolysis of S-adenosyl methionine, whereas S-adenosyl methionine is formed via methylation of methionine [4]. It is reported that serum Hcy can alter the contents of cytoskeleton proteins including G-actin and F-actin, resulting in endothelial cell injury and vascular diseases [5]. Recent studies have shown that hyperhomocysteinemia (HHcy) can induce membrane lipid peroxidation and causes vascular smooth muscle cells (VSMCs) destruction and apoptosis. Meantime, HHcy also promotes VSMCs proliferation, alters coagulation factor function, enhances platelet aggregation, thickens vascular endothelium, and even occludes blood vessels, leading to the risk of CVD through multiple pathways [6-8]. Although serum Hcy closely correlates with the incidence of CVD

such as progressive stroke, recurrent cerebral infarction, and intracerebral hemorrhage (ICH) [9-11], the relationship of laboratory indices (serum Hcy, blood glucose, triglyceride, total cholesterol, and uric acid levels) at hospital admission with the occurrence and prognosis of aSAH has not been revealed.

Hence, a retrospective case-control study on patients with aSAH was performed to analyze the correlation of clinical parameters such as serum Hcy, blood glucose, triglyceride, and blood pressure with disease occurrence and to explore the association between serum Hcy level at admission and poor hospital discharge outcome. The present study results could provide more evidences for aSAH pathogenesis and might aid in the development of prevention strategies.

Material and methods

Patients

From January 1, 2008 to December 31, 2014, a total of 916 inpatients with aSAH at the Neurological Hospital of Logistics University of People's Armed Police Force (China) were screened. Finally, a total of 125 patients (38 males and 87 females) aged between 25 and 83 years (mean age, 57.24 ± 13.06 years) were further enrolled in aSAH group.

Meantime, a total of 1259 cases undergoing physical examination (PE) in our institution were screened to identify the matching healthy subjects. An equivalent healthy subjects (n = 125; 40 males and 85 females) aged between 27 and 83 years (mean age, 55.77 ± 9.72 years) were enrolled in the control group according to the matching sex ratio with aSAH group.

The study protocol was approved by the Hospital Ethics Committee of the Neurological Hospital of Logistics University of People's Armed Police Force, China. The informed consent of the patients and family members was obtained.

Diagnostic criteria

aSAH was diagnosed with reference to the *Guidelines for the Management of aSAH 2012* developed by the American Heart Association/American Stroke Association, which was as follows: 1) acute onset of severe headache, vomiting, and signs of meningeal irritation, with/without loss of consciousness; 2) lumbar puncture showing an elevated intracranial pressure and bloody cerebrospinal fluid; and 3) computed tomography, computed tomography angiography, or digital subtraction angiography (DSA) showing aneurysm.

History of cigarette smoking was defined as ≥ 1 cigarette daily for at least one year based on the World Health Organization (WHO) Standardized Recommendations for Smoking Research Methods 1984.

History of alcohol drinking was defined as 50 g/d or 350 g/w of alcohol consumption lasting for at least six months.

Hypertension was diagnosed with reference to *WHO/International Society of Hypertension Guidelines 1999*, which was as follows: systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg and/or taking antihypertensive drugs.

Serum Hcy ≥ 15 μmol/L was defined as HHcy (reference range: 5~15 μmol/L), which was measured using Hitachi 7180 Biochemistry Automatic Analyzer (Hitachi, Ltd., Tokyo, Japan).

Study inclusion & exclusion criteria

The study inclusion criteria for aSAH group was as follows: 1) aSAH confirmed by DSA (AXIOM Artis BA dBA, Siemens AG, Berlin, Germany) at admission or surgery; 2) serum parameters obtained within the first 24 h after admission (Hitachi 7180 Biochemistry Automatic Analyzer); and 3) the patient's clinical data including demographics, disease course, and detailed laboratory investigations. The study exclusion criteria for aSAH group was as follows: 1) non-aSAH patients with ICH and 2) patients complicated with renal dysfunction, atherosclerosis, stroke, myocardial infarction, or diabetes mellitus.

The study inclusion criteria for control group was as follows: 1) patients undergoing PE with detailed clinical data and 2) healthy subjects (according to the results of PE). The study exclusion criteria for control group was as follows: patients with renal dysfunction, atherosclerosis, stroke, myocardial infarction, or diabetes mellitus as indicated by the PE report.

Methods

A retrospective case-control study was performed using a uniform questionnaire to collect

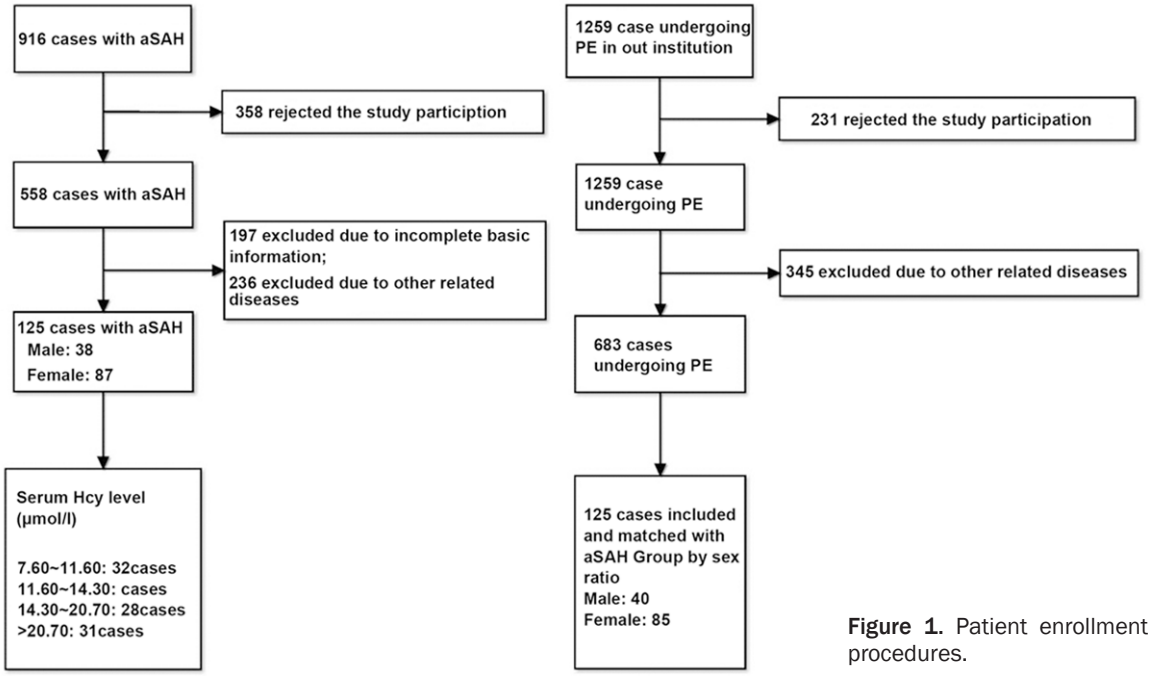

Figure 1. Patient enrollment procedures.

Table 1. Comparison of baseline characteristics of patients at hospital admission between control group and aSAH group

Variables	Control group	aSAH group	$t/\chi^2/z$ value	*P* value
Number of patients	125	125		
Age (years)	55.77 ± 9.725	57.24 ± 13.058	-1.011	0.313
Male [n, (%)]	40 (32.00)	38 (30.40)	0.075	0.785
Cigarette smoking [n, (%)]	23 (18.40)	45 (36.00)	9.777	0.002*
Alcohol drinking [n, (%)]	17 (13.60)	33 (26.40)	6.400	0.011*
History of hypertension [n, (%)]	35 (28.00)	37 (29.60)	0.078	0.780
SBP (mmHg)	130.10 ± 16.121	153.70 ± 27.880	-8.193	< 0.0001*
DBP (mmHg)	84.42 ± 8.838	87.84 ± 15.636	-2.131	0.034*
Uric acid (μmol/L)	307.51 ± 75.634	259.66 ± 112.159	3.955	< 0.0001*
Total cholesterol (mmol/L)	4.30 ± 1.078	4.31 ± 1.435	-0.064	0.949
Triglyceride (mmol/L)$^{\Delta}$	1.45 (0.90)	1.19 (1.705)	-0.543	0.587
Blood glucose (mmol/L)$^{\Delta}$	6.00 (1.20)	7.80 (3.55)	-8.219	< 0.0001*
Hcy (μmol/L)$^{\Delta}$	9.10 (1.30)	14.30 (9.10)	-10.873	< 0.0001*

SBP, systolic blood pressure; DBP, diastolic blood pressure; Hcy, homocysteine; aSAH, aneurysmal subarachnoid hemorrhage. Note: $^{\Delta}$denotes measurement data of skew distribution expressed as median and interquartile range. *denotes $P < 0.05$ as compared the Control group and the aSAH group.

the relevant information, including past disease history, clinical manifestations, present history, PE, imaging examination, and laboratory investigations. The patients' outcomes in aSAH group were scored at hospital discharge by trained attending neurologists according to the Glasgow Outcome Scale (GOS), with good outcome defined as GOS ≥ 4 points while poor outcome as GOS < 4 points. In case of fatal events, if occurred, Hospital Forensic Science Committee opinion would be sought to determine the cause of death and then the death-related information was recorded in the questionnaire. At admission, all the patients in the aSAH group were stratified into four groups using quartile method based on serum Hcy level and if any case with critical Hcy level would be included to the previous rather than the next group. Then the incidence rates of poor outcome were compared between groups of different Hcy levels.

Table 2. Univariate unconditional logistic regression analysis

Variables	OR	95% CI	*P* value
Cigarette smoking	2.495	1.395~4.462	0.002*
Alcohol drinking	2.279	1.192~4.356	0.013*
SBP (mmHg)	1.052	1.036~1.068	< 0.0001*
DBP (mmHg)	1.022	1.001~1.043	0.036*
Uric acid (μmol/L)	0.995	0.992~0.997	< 0.0001*
Blood glucose (mmol/L)	1.900	1.538~2.347	< 0.0001*
Hcy (μmol/L)	1.838	1.545~2.186	< 0.0001*

SBP, systolic blood pressure; DBP, diastolic blood pressure; Hcy, homocysteine; OR, odds ratio; 95% CI, 95% confidence interval. Note: *denotes $P < 0.05$.

Table 3. Multivariate unconditional logistic regression analysis

Variables	OR	95% CI	*P* value
Cigarette smoking	0.834	0.257~2.708	0.762
Alcohol drinking	0.590	0.142~2.444	0.467
SBP (mmHg)	1.112	1.066~1.161	< 0.0001*
DBP (mmHg)	0.919	0.868~0.973	< 0.003*
Uric acid (μmol/L)	0.990	0.984~0.995	< 0.0001*
Blood glucose (mmol/L)	1.548	1.196~2.005	0.001*
Hcy (μmol/L)	2.012	1.555~2.602	< 0.0001*

SBP, systolic blood pressure; DBP, diastolic blood pressure; Hcy, homocysteine; OR, odds ratio; 95% CI, 95% confidence interval. Note: *denotes $P < 0.05$.

Statistical analysis

Statistical analyses of data were performed using SPSS 17.0 software. Normally distributed measurement data were expressed as mean ± standard deviation (SD) and between-group comparison was performed using Student's *t* test. Measurement data of skew distribution were expressed as median and interquartile range and compared using Wilcoxon signed rank-sum test. Count data were expressed as ratio and compared using Chi-square test. Unconditional logistic regression analysis was employed to reveal the aSAH-related risk factors. After stratification of aSAH group into four subgroups based on the serum Hcy level using quartile method, the odds ratio (OR) and 95% confidence interval (95% CI) of the other three groups against the second quartile group (optimal outcome group) were calculated. Two-tailed *P* value was calculated and $P < 0.05$ (marked as *) was considered statistically significant.

Results

Patient enrollment procedures were displayed in **Figure 1.**

Comparison of baseline characteristics between control group and aSAH group

Cigarette smoking, alcohol drinking, SBP, DBP, serum uric acid, blood glucose, and serum Hcy levels were significantly different between the two groups (all $P < 0.05$), whereas the comparison of other factors did not reveal significant differences (all $P > 0.05$) (**Table 1**).

Relationship of Hcy and other laboratory indices with the occurrence of aSAH

Univariate unconditional logistic regression analysis revealed that cigarette smoking, alcohol drinking, SBP, DBP, serum uric acid, blood glucose, and serum Hcy were the risk factors for aSAH (all $P < 0.05$). Then these factors were fitted in multivariate unconditional logistic regression model to identify five risk factors, viz., SBP, DBP, uric acid, blood glucose, and serum Hcy (all $P < 0.05$). After adjusting the other four risk factors (confounding factors), serum Hcy was significantly correlated with the occurrence of aSAH. The overall coefficient of the logistic regression model was significant ($P < 0.05$) and the goodness of fit test (Hosmer-Lemeshow test) results ($P = 0.398 > 0.05$) indicated that the regression equation obtained was acceptable. The results are displayed in **Tables 2** and **3**.

Relationship of serum Hcy level with poor hospital discharge outcome in aSAH group

The incidence rates of poor outcome were significantly varied with serum Hcy levels ($\chi^2 = 8.858$, $P = 0.031$). Chi-square test revealed that the incidence rate of second quartile group (11.60~14.30 μmol/L, group with lowest incidence rate of poor outcome considered as optimal outcome group) was significantly lower than that of the third quartile group (14.30~20.70 μmol/L, $\chi^2 = 4.736$, $P < 0.05$) and the fourth quartile group (> 20.7 μmol/L, $\chi^2 = 8.355$, $P < 0.05$). Further, a J-shape curve was obtained when plotting the incidence rate of poor outcome (Y-axis) against serum Hcy

Table 4. Comparison of patient's outcome between aSAH subgroups of different Hcylevels [n, (%)]

Subgroups (n = 125)	Number of patients	Poor outcome	Good outcome	χ^2 value	*P* value	OR (95% CI)
7.60~11.60	32	11 (34.37)	21 (65.63)	2.412	0.120	2.444 (0.778~7.677)
11.60~14.30	34	6 (17.65)	28 (82.35)	--	--	1.000
14.30~20.70	28	12 (42.86)	16 (57.14)	4.736	0.030*	3.500 (1.101~2.059)
> 20.7	31	16 (51.61)	15 (48.39)	8.355	0.004*	4.978 (1.610~15.387)

Hcy, homocysteine; aSAH, aneurysmal subarachnoid hemorrhage; OR, odds ratio; 95% CI, 95% confidence interval. Note: *denotes $P < 0.05$ as compared with the normal group with serum Hcy level of 11.60~14.30 μmol/L.

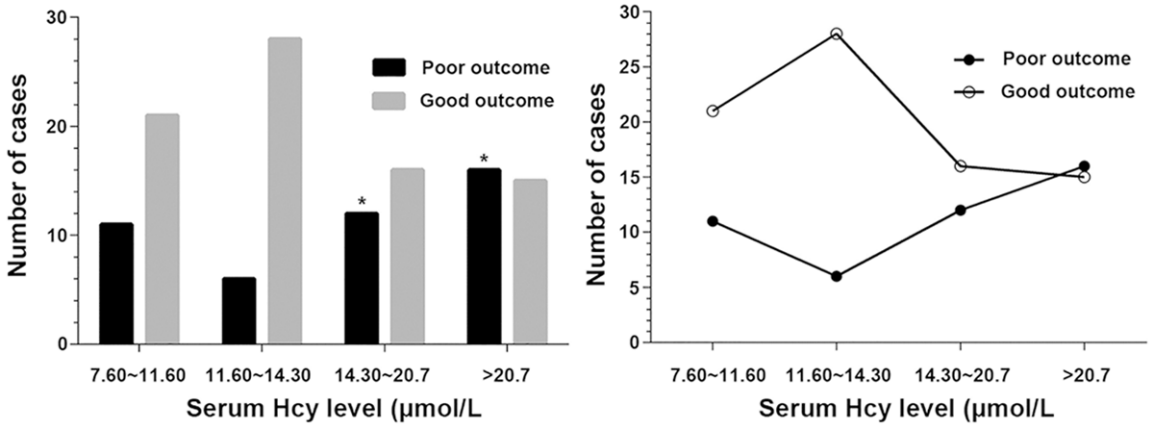

Figure 2. Comparison of patient's outcome between aSAH subgroups of different Hcy levels.

level in aSAH group at hospital admission (X-axis). In relative risk analysis, the first, third, and fourth quartile groups had 2.444-, 3.500-, and 4.978-fold risks for poor outcome compared with the second quartile group. A trend test indicated a linear tendency between the serum Hcy level at admission and the incidence rate of poor hospital discharge outcome in aSAH group ($P = 0.045$). Since one patient in the third quartile group had normal serum Hcy (14.3 μmol/L), all patients in the aSAH group were further classified into either normal or elevated Hcy group for further comparison, which indicated a significant difference regarding the incidence of poor outcome ($\chi^2 = 6.367$, $P = 0.012$). Overall, patients with high serum Hcy level at admission (third plus fourth quartile groups; one patient with normal Hcy level in the third quartile group was insignificant) had 2.603-fold risks for poor outcome when compared to patients with normal serum Hcy level (first plus second quartile groups) and the linear trend was significant (linear trend analysis, $P = 0.012$). In summary, aSAH patients with high serum Hcy level at admission may have poor prognosis at discharge. The results are presented in **Table 4** and **Figure 2**.

Discussion

The present study results revealed that the serum Hcy, SBP, DBP, uric acid, and blood glucose at hospital admission were the independent risk factors for aSAH. On logistic regression analysis, after adjusting the other four risk factors (confounding factors), serum Hcy was significantly correlated with the occurrence of aSAH. Further, aSAH group was divided into four subgroups using quartile method based on serum Hcy level. The Chi-square test results indicated that high serum Hcy level (third + fourth quartile groups) was related to high risks for poor outcome, whereas the second quartile group had the lowest risk for poor outcome. Further, a J-shape curve of the incidence rate of poor outcome against serum Hcy level of patients with aSAH at hospital admission was obtained, which indicated that a high serum Hcy level was not beneficial in terms of progno-

sis of patients with aSAH. A trend test showed a linear tendency between serum Hcy level at admission and the incidence rate of poor hospital discharge outcome in aSAH group. Although the present study is an observational case-control study, a strong association of admission serum Hcy level with the occurrence of aSAH and poor hospital discharge outcome has been reported after excluding other aSAH-related risk factors.

In previous studies, other aSAH-related factors have also been documented. Badjatia *et al.* conducted a prospective cohort trial on 229 patients with aSAH and found that the systemic inflammatory response ensuing from insufficient calorie intake might worsen the negative nitrogen balance and increase the risks for poor prognosis [12]. Meantime, a retrospective case study encompassing 334 patients with aSAH reported the correlation of risk factors (e.g., age, sex, and blood pressure) with poor prognosis [13]. Kurtz *et al.* carried out another retrospective trial which indicated that blood glucose control could have an impact on the cerebral metabolic rate and the prognosis of patients with aSAH [14]. Recently, serum Hcy has been reported to be associated with cardiocerebrovascular events such as hypertension and cerebral infarction. Both the Unal E *et al.* clinical study and the meta-analysis of prospective studies about the correlation of serum Hcy with the risks for different types of stroke (published by He Y 2014) suggested a higher risk for exacerbation of cerebral infarction due to increased serum Hcy levels [15, 16]. Based on a preliminary research that indicated a close relation of the prevalence of H-type hypertension with elevated serum Hcy level in Asian population [17], Huo Y *et al.* performed a large-sample, prospective clinical trial, the China Stroke Primary Prevention Trial [18], which indicated that serum Hcy level might be correlated with stroke-related diseases, including aSAH.

We hypothesize that HHcy is likely to injure the cerebral vessels to induce aSAH and poor prognosis through the following mechanisms. First of all, the serum Hcy with endothelial toxicity causes vascular endothelial cell injury. In 2013, Lee *et al.* discovered that Hcy induced endothelial cell apoptosis by activating the acid sphingomyelinase ceramide pathway in rat [19]. In the same year, Dong D *et al.* conducted a molecular research on human umbilical vein endothelial cells to confirm that serum Hcy decreased the protein levels of copper chaperone COX17, leading to mitochondrial dysfunction and endothelial cell injury [20]. Secondly, serum Hcy can directly induce the proliferation of VSMCs. In 2012, it was reported that high-concentration of serum Hcy activated the VSMCs through deoxyribonucleic acid demethylation of platelet-derived growth factors in endothelial cells, resulting in VSMCs proliferation [21]. Lastly, serum Hcy reduces the antithrombogenic factor to promote platelet adhesion, aggregation, and thus thrombus formation [22]. The above three mentioned mechanisms related to vascular injury may underpin the pathologic vascular changes in aSAH. On the basis of exacerbated vascular injury caused by persistent high-levels of serum Hcy, a hemodynamic change such as increased blood pressure (especially a sustained high SBP) causes vascular cell apoptosis and extensive disruption of the elastic layer, leading to protuberance on the vascular wall, namely aneurysm. Consequently, it may lead to risks for aSAH with possible poor outcome. Hence, in summary, a high serum Hcy level may be a risk factor for aSAH and may lead to poor prognosis. In brief, patients with normal serum Hcy level are less likely to suffer from a poor prognosis as compared with those with HHcy.

However, the present study has certain potential limitations. During data analysis, high risks for poor outcome were observed in the first quartile group which had a lower serum Hcy level than the second quartile group, although both groups had normal Hcy levels. The phenomenon which was not significant as indicated by the chi-square test remains unclear because of a limited sample size. Hence, further clinical studies with larger sample size are warranted to identify the significance of low serum Hcy level on patients with aSAH in relation with their prognosis. Moreover, one patient with normal serum Hcy (14.4 μmol/L) stratified under third quartile group could possibly influenced the stratification process and significantly impacted the linear regression analysis and trend test results. As laboratory indices at hospital admission were only considered in this observational study, more controlled prospective trials should be needed to determine the relationship between serum Hcy level and the patient's prognosis at different periods of hospitalization.

Conclusions

In conclusion, the present study results indicate that the serum Hcy level at hospital admission could be an important independent risk factor for the occurrence and prognosis of aSAH. Moreover, a J-shape curve between serum Hcy level at admission and the incidence rate of poor hospital discharge outcome has also been established. Hence, modifying the serum Hcy level in an appropriate way is probably important for the prevention and treatment of aSAH; however, further studies are required to support the present study findings.

Acknowledgements

This study was partially supported by the Science and Technology Fund of Health Broad of Tianjin (Grant No. 2015KZ126), The Fund of Health and Family Planning Commission of Tianjin (Grant No. 2015155), The Fund of Tianjin Key Laboratory of Neuro-trauma Repair (Grant No. WYKFZ201602).

Disclosure of conflict of interest

None.

Address correspondence to: Zhihong Wang, Postgraduate Training Base, Affiliated Hospital of Logistics University of Chinese People's Armed Police Force, Jinzhou Medical University, No. 220, Chenglin Road, Hedong District, Tianjin 300162, China. Tel: +86-18822391996; Fax: +86-022-60578188; E-mail: wujingwzh@sina.com; Shengkai Sun, Department of Medical Education of CAPF, No. 220, Chenglin Road, Hedong District, Tianjin 300162, China. Tel: +86-18822391996; Fax: +86-022-60578188; E-mail: sskrui@163.com

References

[1] da Costa L, Fisher J, Mikulis DJ, Tymianski M and Fierstra J. Early identification of brain tissue at risk for delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage. Acta Neurochir Suppl 2015; 120: 105-109.

[2] Sundboll J, Schmidt M, Horvath-Puho E, Christiansen CF, Pedersen L, Botker HE and Sorensen HT. Impact of preadmission treatment with calcium channel blockers or beta blockers on short-term mortality after stroke: a nationwide cohort study. BMC Neurol 2015; 15: 24.

[3] Juvela S, Poussa K, Lehto H and Porras M. Natural history of unruptured intracranial aneurysms: a long-term follow-up study. Stroke 2013; 44: 2414-2421.

[4] Barnabe A, Alessio AC, Bittar LF, de Moraes Mazetto B, Bicudo AM, de Paula EV, Hoehr NF and Annichino-Bizzacchi JM. Folate, vitamin B12 and Homocysteine status in the post-folic acid fortification era in different subgroups of the Brazilian population attended to at a public health care center. Nutr J 2015; 14: 19.

[5] Zhang YY, Shen W, Zhang LC, Pan ZY, Long CL, Cui WY, Zhang YF and Wang H. Proteomics reveals potential non-neuronal cholinergic receptor-effectors in endothelial cells. Acta Pharmacol Sin 2014; 35: 1137-1149.

[6] Catena C, Colussi G, Nait F, Capobianco F and Sechi LA. Elevated Homocysteine Levels Are Associated With the Metabolic Syndrome and Cardiovascular Events in Hypertensive Patients. Am J Hypertens 2015; 28: 943-950.

[7] Catena C, Colussi G, Nait F, Capobianco F and Sechi LA. Plasma lipoprotein(a) levels and atherosclerotic renal artery stenosis in hypertensive patients. Kidney Blood Press Res 2015; 40: 166-175.

[8] Catena C, Colussi G, Url-Michitsch M, Nait F and Sechi LA. Subclinical carotid artery disease and plasma homocysteine levels in patients with hypertension. J Am Soc Hypertens 2015; 9: 167-175.

[9] Kwon HM, Lee YS, Bae HJ and Kang DW. Homocysteine as a predictor of early neurological deterioration in acute ischemic stroke. Stroke 2014; 45: 871-873.

[10] Fu HJ, Zhao LB, Xue JJ, Wu ZX, Huang YP, Liu W and Gao Z. Elevated Serum Homocysteine (Hcy) Levels May Contribute to the Pathogenesis of Cerebral Infarction. J Mol Neurosci 2015; 56: 553-561.

[11] Zhou F, Chen B, Chen C, Huang J, Chen S, Guo F and Hu Z. Elevated homocysteine levels contribute to larger hematoma volume in patients with intracerebral hemorrhage. J Stroke Cerebrovasc Dis 2015; 24: 784-788.

[12] Badjatia N, Monahan A, Carpenter A, Zimmerman J, Schmidt JM, Claassen J, Connolly ES, Mayer SA, Karmally W and Seres D. Inflammation, negative nitrogen balance, and outcome after aneurysmal subarachnoid hemorrhage. Neurology 2015; 84: 680-687.

[13] Rivero Rodriguez D, Scherle Matamoros C, Fernandez Cue L, Miranda Hernandez JL, Pernas Sanchez Y and Perez Nellar J. Factors associated with poor outcome for aneurysmal subarachnoid haemorrhage in a series of 334 patients. Neurologia 2015; [Epub ahead of print].

[14] Kurtz P, Claassen J, Helbok R, Schmidt J, Fernandez L, Presciutti M, Stuart RM, Connolly ES, Lee K, Badjatia N and Mayer SA. Systemic glucose variability predicts cerebral metabolic distress and mortality after subarachnoid hemorrhage: a retrospective observational study. Crit Care 2014; 18: R89.

[15] Unal E, Mungan S, Bilen S, Karadag Y, Oztekin N, Bakir F and Ak F. The effects of lipoprotein(a) and homocysteine on prognosis and risk factors in acute ischemic stroke. Int J Neurosci 2013; 123: 532-536.

[16] He Y, Li Y, Chen Y, Feng L and Nie Z. Homocysteine level and risk of different stroke types: a meta-analysis of prospective observational studies. Nutr Metab Cardiovasc Dis 2014; 24: 1158-1165.

[17] Wang Y, Li X, Qin X, Cai Y, He M, Sun L, Li J, Zhang Y, Tang G, Wang B, Sun N, Xu X, Liu L, Xu X and Huo Y. Prevalence of hyperhomocysteinaemia and its major determinants in rural Chinese hypertensive patients aged 45-75 years. Br J Nutr 2013; 109: 1284-1293.

[18] Huo Y, Li J, Qin X, Huang Y, Wang X, Gottesman RF, Tang G, Wang B, Chen D, He M, Fu J, Cai Y, Shi X, Zhang Y, Cui Y, Sun N, Li X, Cheng X, Wang J, Yang X, Yang T, Xiao C, Zhao G, Dong Q, Zhu D, Wang X, Ge J, Zhao L, Hu D, Liu L and Hou FF. Efficacy of folic acid therapy in primary prevention of stroke among adults with hypertension in China: the CSPPT randomized clinical trial. JAMA 2015; 313: 1325-1335.

[19] Lee JT, Peng GS, Chen SY, Hsu CH, Lin CC, Cheng CA, Hsu YD and Lin JC. Homocysteine induces cerebral endothelial cell death by activating the acid sphingomyelinase ceramide pathway. Prog Neuropsychopharmacol Biol Psychiatry 2013; 45: 21-27.

[20] Dong D, Wang B, Yin W, Ding X, Yu J and Kang YJ. Disturbance of copper homeostasis is a mechanism for homocysteine-induced vascular endothelial cell injury. PLoS One 2013; 8: e76209.

[21] Zhang D, Chen Y, Xie X, Liu J, Wang Q, Kong W and Zhu Y. Homocysteine activates vascular smooth muscle cells by DNA demethylation of platelet-derived growth factor in endothelial cells. J Mol Cell Cardiol 2012; 53: 487-496.

[22] Glueck CJ, Smith D, Gandhi N, Hemachandra K, Shah P and Wang P. Treatable high homocysteine alone or in concert with five other thrombophilias in 1014 patients with thrombotic events. Blood Coagul Fibrinolysis 2015; 26: 736-42.