Bull Yamaguchi Med Sch 49(3-4):81-86, 2002

Elevated Plasma Homocysteine is One of the Risk Factors for Sudden Cardiac Death in Japanese

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(Received December 27, 2002, revised January 22, 2003)

Key words: Cardiac arrest, Cardiopulmonary resuscitation (CPR)
Out-of-hospital CPR

Abstract *Objective* Hyperhomocysteinemia has been reported to be an independent risk factor for myocardial infarction and death in coronary artery disease in abroad. To investigate the clinical significance of plasma homocysteine in Japanese sudden cardiac death, we assessed its levels in cardiopulmonary arrest victims caused by cardiac etiology.

Methods Plasma homocysteine was determined by a high-performance liquid chromatograph with a fluorometric detector.

Patients Homocysteine levels were assessed in 41 cardiopulmonary arrest (CPA) victims and in 104 healthy control subjects. The CPA patients were classified in the three groups based on the etiology, i.e., cardiogenic, noncardiogenic (such as bronchial asthma and subarachnoid hemorrhage), or accidental (such as asphyxia and hanging) CPA group.

Results Plasma homocysteine in cardiogenic CPA patients was higher than that in noncardiogenic and accidental CPA patients, or in healthy control subjects. Plasma homocysteine in healthy men was higher than that in healthy women. Additionally, homocysteine levels decreased progressively with age in healthy subjects ($p<0.05$).

Conclusion These results demonstrated that high plasma homocysteine is a significant risk factor for sudden cardiac death and that a warning is inevitable for male younger generation in Japanese.

Introduction

Homocysteine is a sulfur-containing amino acid, produced by methionine metabolism. The 1996 Bethesda Conference acknowledged possible risk factors for coronary artery disease, which were left ventricular hypertrophy, hyperhomocysteinemia, lipoprotein excess, hypertriglyceridemia, hyperfibrinogemia, and oxidative stress[1]. Although the mechanism of atherosclerosis induced by hyperhomocysteinemia is not fully understood, elevated plasma homocysteine has been reported as a risk factor for coronary artery disease[2,3]. Furthermore, even mild to moderate hyperhomocysteinemia is independently associated with mortality in patients with coronary artery disease[4]. On the other hand, Alfthan et al. reported inter-country differences in homocysteine levels[5]. According to their pa-

Table 1 Characteristics of healthy volunteers and cardiopulmonary arrest patients

Characteristics	Healthy volunteers (n=18)	Cardiogenic CPA (n=19)	Noncardiogenic CPA (n=11)	Accidental CPA (n=11)	p value
Age (yr)	58±1	60±2	64±3	60±3	0.27
Sex (%male)	61.1	63.2	63.6	90.9	0.34
Collapse-to-start of CPR interval (min)	−	9±3	16±4	22±7	0.14

Plus-minus values are means ±SE.
CPA; cardiopulmonary arrest, CPR; cardiopulmonary resuscitation

per, both plasma homocysteine and mortality of cardiovascular disease in Japan were much lower than those in Finland, Scotland, or Northern Ireland, although these data were obtained between 1983 and 1990. In last decade, Japanese has taken western diet, containing more methionine and less vitamins. Therefore, we hypothesized that plasma homocysteine would increase in last decade and contribute to mortality by coronary artery disease in Japanese.

Methods

Subjects

Between May 1999 and January 2001, blood samples were drawn from 48 cardiopulmonary arrest (CPA) victims (over 50 years old) who were brought into the emergency room at our university hospital by ambulances. The study protocol was approved by the Institutional Review Board of our university hospital. Subjects were excluded if they had a history of chronic renal failure, malignancy or were taking medications that might influence plasma homocysteine (e.g., methotrexate, anticonvulsants). The subjects were classified into three groups based on the etiology of cardiac arrest, which was determined by the information of medical history, blood examination, cerebrospinal fluid or CT scans. The characteristics of CPA patients and healthy volunteers are given in Table 1. Cardiogenic CPA (n=19) was defined by cardiac etiology which was determined by the medical records of heart disease, elevated serum creatine kinase and/or positive troponin T test (Roche diagnostics, Switzerland). The causes of noncardiogenic CPA were subarachnoid hemorrhage (n=5), cerebral hemorrhage (n=3), bronchial asthma (n=1), gastrointestinal bleeding (n=1), and pulmonary fibrosis (n=1). The causes of accidental CPA were hanging (n=4), asphyxia (n=4), carbon monoxide poisoning (n=1), cervical spinal injury (n=1), and drowning (n=1). Seven subjects were excluded, because their cause of death was unknown. All CPA patients were dead except one, who was finally in vegetative state.

We measured plasma homocysteine of 104 healthy volunteers during fasting in the same area, where CPA patients were delivered to us. The healthy volunteers consisted of both sexes, who underwent annual health care and informed consent was obtained from all of them. Healthy volunteers over 50 years old were selected to adjust the age of CPA patients (Table 1). To determine whether plasma homocysteine changed during cardiopulmonary resuscitation, plasma homocysteine in accidental CPA patients was used as the control to compare to that in healthy volunteers, which was not changed.

Blood Sample Collection

Two milliliter of venous or arterial blood was drawn from each CPA patient on arrival or healthy volunteer, mixed with ethylenediaminetetraacetate (EDTA) in the evacuated tube and kept at 4°C. The blood samples were centrifuged at 3,000×g for 10 minutes at 4°C within 2 hours. The plasma was stored at -80°C until analysis.

Homocysteine measurement

Total plasma homocysteine (sum of

Table 2 Plasma homocysteine in healthy volunteers

Age (yr)	20-29	30-39	40-49	50-59	Total
Men (μmol/L)	18.1±5.0	11.4±1.5	11.2±2.0	9.8±0.8	12.9±1.6*
	(n=15)	(n=14)	(n=13)	(n=11)	(n=53)
Women (μmol/L)	7.5±0.6	6.9±0.4	6.3±0.2	5.8±0.3	6.6±0.2
	(n=9)	(n=13)	(n=22)	(n=7)	(n=51)

Plus-minus values are means ±SE.
*; Significantly different from women ($p<0.01$). Plasma homocysteine decreased progressively with age ($p<0.05$, by ANOVA and Williams' test)

protein-bound and free homocysteine) was determined by a high-performance liquid chromatograph (L-7000, Hitachi instruments service, Japan) with a fluorometric detector (L-7480, Hitachi instruments service, Japan), based on the method of Ubbink et al[6].

Statistic analysis

Data are presented as mean ± SE. Student's t-test was used for paired comparison and one-way ANOVA was used for comparison of more than two variables, to determine statistical significance. When significant differences were observed between the groups, comparisons were made by Dunnett's test or Williams' test. Significance was accepted at $p<0.05$.

Results

Plasma homocysteine in CPA patients

Homocysteine levels in cardiogenic CPA patients (13.4±2.4 μmol/L) were higher than those in noncardiogenic CPA patients (7.9±0.6 μmol/L) and accidental CPA patients (8.1±0.7 μmol/L), or in healthy volunteers (8.2±0.7 μmol/L) (Figure 1).

Plasma homocysteine in healthy volunteers

Homocysteine levels were significantly higher in men than in women (12.9 ± 1.6 vs 6.6 ± 0.2 μmol/L) and decreased pro gressively with age (Table 2). The distributions of homocysteine levels are shown in Figure 2. Eight men (15% of men) had hyperhomocysteinemia, whose levels were more than 15.0 μmol/L. Their levels were 67.0, 56.1, 37.5, 31.9, 29.0, 18.9 16.0 and 16.0 μmol/L, whereas the highest level in women was 13.2 μmol/L. The highest 3 homocysteine levels were recorded in men 20-29 years old.

Discussion

Hyperhomocysteinemia has not been well

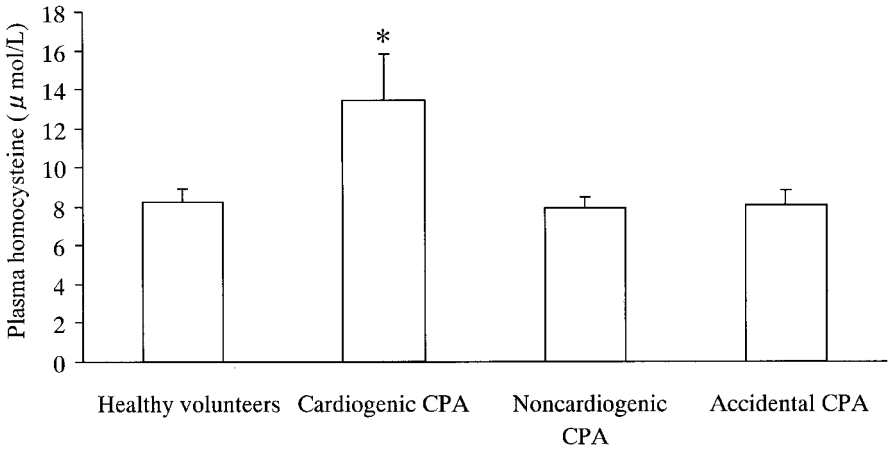

Fig. 1 Plasma homocysteine in healthy volunteers, cardiogenic, noncardiogenic and accidental CPA patients.
Data are presented as mean ±SE. *Significantly different from healthy volunteers ($p<0.05$, by ANOVA and Dunnett's test)

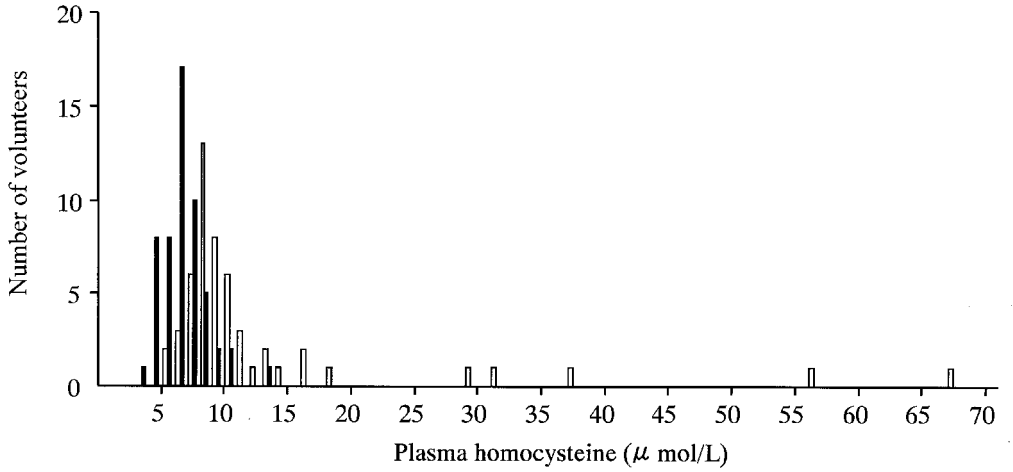

Fig. 2 Distribution of plasma homocysteine in healthy men (open bars) and women (closed bars).

recognized and a few papers refer the relationship between hyperhomocysteinemia and coronary artery disease in Japan[7,8,9]. Our present study clearly demonstrates that plasma homocysteine in cardiogenic CPA patients is higher than that in noncardiogenic or accidental CPA patients (Figure 1). Relatively higher plasma homocysteine could induce asymptomatic/inapparent coronary artery disease and finally induce CPA. Actually, Nygard et al. reported that plasma homocysteine was a strong predictor of mortality for coronary artery disease in Norwegian[4].

Cardiogenic CPA has been based on a diagnosis of exclusion and infrequently determined by autopsy data and hospital records[10]. In our study, 3 out of 19 cardiogenic CPA patients were obviously diagnosed as acute myocardial infarction by echocardiography, coronary angiography and/or autopsy. The other 16 patients were diagnosed by elevated serum creatine kinase, positive troponine T test, and the medical records of heart disease. None of them were diagnosed by the exclusion of noncardiogenic etiology. The causes of noncardiogenic or accidental CPA are often obvious and easy to determine, such as drug overdose, suicide, drowning, hypoxia, exsanguination, subarachnoid hemorrhage, and trauma[10]. Therefore, the grouping for our cardiogenic CPA is much more apparent than the previous reports.

Kang et al. measured plasma homocysteine during fasting and classified hyperhomocysteinemia as moderate (15 to 30 μ mol/L), intermediate (>30 to 100 μ mol/L), and severe (>100 μ mol/L) levels[11]. In a typical western diet, a postprandial increase in plasma homocysteine is usually small (1 to 2 μ mol/L)[12]. In our cardiogenic CPA patients, there was no patient belonging to the severe levels and only 2 patients were belonging to the intermediate levels, even if our patients would be considered nonfasting state. These results indicate that Japanese plasma homocysteine is not extremely high as that of western patients and the contribution of hyperhomocysteinemia to cardiogenic CPA would be much less than that in western people at this stage.

In the present study, plasma homocysteine in healthy volunteers is markedly higher in men than in women (Table 2), which is agreeable to previous reports[13,14]. The difference may come from sex hormones. However, even at age 50 to 59 years, men had higher levels than women, which is suggesting additional mechanisms such as nutritional factors. Homocysteine is metabolized by two pathways: vitamin B_6-dependent transsulfuration, and vitamin B_{12}-dependent or folate-dependent remethylation. In homocystinuria caused by deficiency of the vitamin B_6-dependent enzyme (cystathionine β-synthase), plasma homocysteine is markedly elevated and patients have severe, widespread vascular

disease[15]. However, mild to moderate elevations in plasma homocysteine are common and may be due to inherited enzyme variants and/or a nutritional deficiency of folate, vitamin B_{12} or vitamin B_6[16,17]. In this study, genetic factors were not investigated. Although plasma homocysteine increased progressively with age in both sexes[13,14], peak of homocysteine levels in the present study was recorded in men 20-29 years old. We speculated that younger men might have nutritional problems, which may come from deficiencies of folate, vitamin B_{12}, or vitamin B_6[17]. When the present two results are concerned together, i.e., significantly higher plasma homocysteine in cardiogenic CPA patients (over 50 years old) and in healthy younger men (20-29 years old), a hypothesis could be led that the younger Japanese men would become a seriously risky group for coronary artery disease within 30 years. To prevent the risk in these hyperhomocysteinemia volunteers, they should be treated.

Burke et al. have recently demonstrated that serum homocysteine is elevated in sudden death as a result of severe coronary artery disease without thrombosis[18]. The possible mechanism may be endothelial dysfunction, which is improved by folate and vitamin B_{12} supplementation[19]. Large randomized trials are in progress to determine whether multivitamine therapy to decrease plasma homocysteine will reduce the risk for cardiovascular disease[20]. When a combination of vitamins is found to be effective, we must inform the results to those volunteers to prevent cardiovascular disease.

In conclusion, the elevated plasma homocysteine is a risk factor for sudden cardiac death in aged generation (over 50 years old) and would be in healthy younger men (20-29 years old) in Japanese. Further investigations are needed to clarify the biological significance of homocysteine in the pathogenesis in coronary artery disease.

Acknowledgements

I greatly thank Professor Tsuyoshi Maekawa for preparing this manuscript and Mrs. Hitomi Ikemoto for the technical assistance.

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