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Original Article

Relationship between increase of serum homocysteine caused by smoking and oxidative damage in elderly patients with cardiovascular disease

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Abstract: Background: To investigate the mechanism of smoking on cardiovascular diseases. Material and methods: 576 elderly patients with cardiovascular diseases in stable condition were consecutive recruited, asked about the living habits and smoking history in a way of face to face. Results: Of all the enrolled patients, current smoking rate was 34.8% for males and 3.4% for females. Average smoking quantity was 17 cigarettes per day and incidence of hyperhomocysteinemia was 38.0%. The homocysteine level in current smokers was significantly higher than that in never smokers ($P = 0.004$); while the serum folic acid and serum superoxide dismutase (SOD) level were significantly lower those in never smokers ($P = 0.012$; $P = 0.004$). The daily smoking consumption and the pack-years of smoking were significantly positively correlated with total homocysteine (tHcy) level ($P = 0.020$; $P = 0.003$). The reduced serum SOD level might be associated with increased risk of hypertension ($P = 0.023$), coronary heart disease ($P = 0.018$), and stroke ($P = 0.035$). However, the elevated serum tHcy level was not correlated with increased risk of hypertension and coronary heart disease, while may increase the risk of ischemic stroke ($P = 0.075$). Conclusions: Smoking status is still prevalent among Chinese elderly patients with cardiovascular diseases, which causes the increase of serum tHcy and the decrease of serum folate as well as SOD; smoking consumption per day and pack-years of smoking have indirect effects on tHcy. And decrease of serum SOD is a risk factor for cardiovascular diseases, increase of serum tHcy may be associated with changes of metabolism caused by oxidative damage.

Keywords: Cardiovascular disease, homocysteine, cigarette smoking, superoxide dismutase

Introduction

Cardiovascular disease is the leading threat to human health as well as the major cause of death worldwide, especially in developed countries. Currently, there are about 2.9 million patients with cardiovascular disease in China [1], in a rising trend. It was estimated that the annual economic burden caused by cardiovascular disease toped approximately $ 550 billion. Many studies have proved that smoking, except as a risk factor for respiratory tract neoplasms and chronic obstructive pulmonary diseases, was an independent risk factor of cardiovascular diseases, and could increase the incidence of myocardial infarction and fatal coronary artery diseases [2-4]. Cigarette smoke contains toxic chemicals [5] with large amounts of oxidants both in the gas phase and particle phase, which, through the generation of active oxygen system and free radicals, affects the body's antioxidant defense system.

Homocysteine (Hcy) is an intermediate product of methionine metabolism. When the upstream or downstream metabolic pathway of homocysteine is blocked or delayed for various reasons, it will lead to the accumulation of homocysteine, causing hyperhomocysteinemia (HHcy). It has been confirmed that the increase of Hcy was an independent risk factor for vascular diseases, including coronary artery disease, cerebrovascular disease, and peripheral vascular obstructive lesions [6, 7]. And studies found that heredity, nutrition, lifestyle and environmental factors affect total homocysteine (tHcy) concentration [8, 9]. In recent years, a growing number of studies have suggested that the elevated serum Hcy was one of the important markers of cardiovascular diseases.

Over past years, more and more studies focused on the effects of smoking on such traditional cardiovascular risk factors as blood pressure and lipid profiles [10-12], while the studies of novel risk factors caused by smoking were less published. Taking the elderly patients with cardiovascular diseases as participants, we tested the impact of smoking on serum biochemical indexes in these patients, in order to investigate the mechanism of smoking on cardiovascular diseases.

Materials and methods

Participants

576 participants with cardiovascular diseases in stable condition were consecutively recruited from the medical wards of Shanghai Tongji Hospital of Tongji University from June 2012 to June 2014. Inclusion criteria: 1) age ≥ 60 years; 2) no serious organ failure; 3) no acute infection, trauma; 4) no chronic gastrointestinal disease. Exclusion criteria: 1) regular Vitamin B users; 2) vegetarians. The study was approved by Medical College of Tongji University Ethics Committee.

Smoking questionnaire

A questionnaire was conducted on every participant by face-to-face interview, to obtain their smoking status by asking whether they smoked or not. If the answer was “yes”, further information was needed to provide on the duration of smoking and the number of cigarette smoked per day; if the answer was “no”, following questions will be asked: “Do you never smoke or quit smoking?”, “How many years have you quit smoking?”. “Smoking” was defined by answers of having smoked more than 100 cigarettes in participant’s lifetime, almost having smoked at least 3 to 4 consecutive months or having smoked one year or more altogether. “Current smoker” was defined as the participants was in cigarette smoking or smoked within one month before investigation; “former smoker” referred to the participants who ever smoked and quitted smoking for at least 3 months. “Heavy smoker” was defined as the participants smoked 20 cigarettes or more cigarettes per day; “cigarette per day” was defined as the average number of cigarettes smoked per day of current smokers; “pack-years of smoking” equals to the average number of packs of cigarettes smoked per day multiplying the number of years of smoking.

Biochemical measurements

The venous blood samples were drawn from fasting participants (more than 8 hours) in the next morning after admission. Serum tHcy was measured by using enzymatic cycling method, (normal reference range: < 15 μmol/l); serum superoxide dismutase (SOD) was tested by colorimetry (normal reference range: 129-216 U/ml). Determination of lipids was made through enzymatic or chemical method. The normal reference range of total cholesterol (TC) is 3.1-5.2 mmol/l, low-density lipoprotein (LDL) lees than 3.12 mmol/l, high-density lipoprotein (HDL) more than 0.91 mmol/l, triglycerides (TG) less than 1.7 mmol/l. Prealbumin (PA) was tested by using transmission turbidimetry (normal reference range: 0.20-0.48 g/l); serum albumin (ALB), uric acid (UA), creatinine (Cr) were observed by applying chemical methods, with the reference ranges of 40-55 g/l, 155-420 μmol/l and 62-115 μmol/l, respectively. Glucose (Glu) was tested with an enzymatic electrode method (normal reference range: 3.9-6.1 mmol/l); glycated hemoglobin A1c (HbA1c) was analyzed with high performance liquid chromatography (normal reference range: 4.2-5.9%). Serum folate and vitamin B_{12} (VB_{12}) were measured by electro-chemiluminescence assay, with the normal reference ranges of 10.4-42.4 nmol/l and 141-489 pmol/l, respectively.

Statistical analysis

The normality of the data distribution was examined by Kolmogorov-Smirnov test. The normality variables were expressed as mean ± SD, and asymmetric variables were presented as geometric mean ± SD and analyzed after log-transformation. Differences between groups of smoking status were analyzed with ANOVA, and the general linear model was used to correct confounding factors. Pairwise comparison was performed among current smokers, never smokers and former smokers using LSD test. Logistic regression analysis was used to investigate the risk factors of cardiovascular diseases and Partial correlation was used to look at the relationships between smoking status and biochemical indexes. Categorical variables were expressed as percentages, and dif-

Table 1. The characteristics of participants

	Total (n = 576)	Men (n = 256)	Women (n = 320)	*P*
Age (y)*	75.6 ± 8.5	73.5 ± 8.9	77.2 ± 7.9	0.000
SBP (mmHg)*	139 ± 21	139 ± 21	140 ± 21	0.427
DBP (mmHg)*	80 ± 11	81 ± 12	78 ± 10	0.001
Body mass index (kg/m^2)*	24.2 ± 3.9	24.1 ± 3.7	24.3 ± 4.0	0.678
Level of education (n, %)**				
Primary or below	266 (46.2)	69 (26.9)	197 (61.6)	0.000
Secondary school	230 (40.0)	132 (51.6)	98 (30.6)	0.000
College or above	80 (13.8)	55 (21.5)	25 (7.8)	0.000
Smoking status (n, %)**				
Never smoker	397 (68.9)	94 (36.7)	303 (94.7)	0.000
Former smoker	79 (13.7)	73 (28.5)	6 (1.9)	0.000
Current smoker	100 (17.4)	89 (34.8)	11 (3.4)	0.000
Alcohol consumption (n, %)**				
Nondrinker	518 (89.9)	202 (78.9)	316 (98.8)	0.000
< 1 drink/d	11 (1.9)	10 (3.9)	1 (0.3)	0.002
≥ 1 drink/d	47 (8.2)	44 (17.2)	3 (0.9)	0.000
Physical activity (n, %)**				
Sedentary lifestyle	342 (59.4)	133 (52.0)	209 (65.3)	0.001
Recreational activity	234 (40.6)	123 (48.0)	111 (34.7)	0.001
Distribution of disease (n, %)**				
Hypertension	447 (77.6)	183 (71.5)	264 (82.5)	0.002
Ischemic stroke	213 (37.0)	108 (42.2)	105 (32.8)	0.021
Coronary heart disease	313 (54.3)	130 (50.8)	183 (57.2)	0.125
Diabetes	200 (34.7)	79 (30.9)	121 (37.8)	0.082
Hcy (umol/l)***	14.61 ± 1.57	16.07 ± 1.60	13.54 ± 1.52	0.000
Folate (nmol/l)***	20.39 ± 1.66	17.19 ± 1.64	23.69 ± 1.59	0.000
VB_{12} (pmol/l)***	333.6 ± 2.2	311.1 ± 2.1	354.2 ± 2.4	0.163
SOD (U/l)****	119.1 ± 22.1	120.4 ± 23.6	118.1 ± 20.9	0.215
CRP(mg/l)***	5.53 ± 2.65	5.99 ± 2.87	5.21 ± 2.45	0.108
Glu (mmol/l)***	5.74 ± 1.32	5.81 ± 1.31	5.69 ± 1.33	0.383
HbA1c (%)***	6.66 ± 1.24	6.62 ± 1.25	6.69 ± 1.22	0.633
UA (μmol/l)***	323.8 ± 1.4	343.8 ± 1.3	311.1 ± 1.4	0.000
Cr (μmol/l)***	80.6 ± 1.4	90.9 ± 1.3	73.7 ± 1.5	0.000
Alb (g/L)****	37.7 ± 3.9	37.7 ± 4.2	37.8 ± 3.6	0.745
PA (g/L)****	0.26 ± 0.04	0.26 ± 0.04	0.25 ± 0.04	0.103
TC (mmol/l)****	4.42 ± 1.10	4.19 ± 1.04	4.60 ± 1.11	0.000
TG (mmol/l)****	1.50 ± 0.94	1.40 ± 0.91	1.59 ± 0.95	0.013
HDL-C (mmol/l)****	1.12 ± 0.30	1.05 ± 0.28	1.18 ± 0.31	0.000
LDL-C (mmol/l)****	2.53 ± 0.86	2.44 ± 0.83	2.61 ± 0.88	0.018

Footnotes: SBP, Systolic blood pressure; DBP, Diastolic blood pressure; Hcy, Homocysteine; SOD, oxide dismutase; CRP, C-reactive protein; Glu, Fasting blood glucose; UA, Uric acid; Cr, Creatinine, PA, Prealbumin; 1 drink, equivalent to drinking 15 g alcohol. Recreational activity, such as jogging, brisk walking, bicycling, shadow boxing, or body-building dance. *Continuous variable comparison between men and women by student's t-test. **Classification variables comparison between men and women by Chi-square test. ***Continuous variable of asymmetry distribution data by the natural logarithm transformation analysis, described as a geometric mean ± standard deviation, comparison between men and women by independent sample t test. ****Continuous variable of normal distribution data described as mean ± standard deviation, comparison between men and women by independent sample t test.

Table 2. Smoking status and clinical characteristics of participants

	Men (n = 256)			Women (n = 320)		
	Current smoker (n = 89)	Former smoker (n = 73)	Never smoker (n = 94)	Current smoker (n = 11)	Former smoker (n = 6)	Never smoker (n = 303)
Age (year)						
60-69 y	50 (56.2)*	25 (34.2)	28 (29.8)	1 (9.1)	1 (16.7)	62 (20.5)
70-79 y	19 (21.3)	30 (41.1)	26 (27.7)	3 (27.3)	2 (33.3)	99 (32.7)
≥ 80 y	20 (22.5)	18 (24.7)	40 (42.5)**	7 (63.6)	3 (50.0)	142 (46.9)
Blood pressure (mmHg)						
Systolic pressure	137 ± 19	137 ± 22	142 ± 23	140 ± 21	141 ± 14	146 ± 24
Diastolic pressure	80 ± 11	80 ± 11	84 ± 13***	78 ± 11	82 ± 12	79 ± 10
Level of education (n, %)						
Primary or below	24 (27.0)	20 (22.5)	25 (26.6)	9 (81.8)	5 (83.3)	185 (61.1)
Secondary school	52 (58.4)	32 (40.4)	44 (46.8)	2 (18.2)	1 (16.7)	95 (31.4)
College or above	13 (14.6)	17 (19.1)	25 (26.6)	0	0	23 (7.5)
Body mass index (n, %)						
Underweight	7 (7.9)	2 (2.7)	10 (10.6)	2 (18.2)	1 (16.7)	21 (6.9)
Normal weight	34 (38.2)	28 (38.4)	39 (41.5)	5 (45.5)	1 (16.7)	130 (42.9)
Overweight	36 (40.4)	26 (35.6)	34 (36.2)	2 (18.2)	3 (50.0)	95 (31.4)
Obesity	22 (13.5)	17 (23.3)	11 (11.7)	2 (18.2)	1 (16.7)	57 (18.8)
Alcohol consumption (n, %)						
Nondrinker	52 (58.4)	62 (84.9)	88 (93.6)*	9 (81.8)	6 (100)	301 (99.3)
< 1 drink/d	6 (6.8)	3 (4.1)	1 (1.1)	1 (9.1)	0	0
≥ 1 drink/d	31 (34.8)	8 (11.0)	5 (5.3)	1 (9.1)	0	2 (0.7)
Physical activity (n, %)						
Sedentary lifestyle	50 (56.2)	35 (47.9)	48 (51.1)	9 (81.8)	6 (100)	194 (64.0)
Recreational activity	39 (43.8)	38 (52.1)	46 (48.9)	2 (18.2)	0	109 (36.0)
Distribution of disease (n, %)						
Hypertension	66 (74.2)	48 (65.8)	69 (73.4)	9 (81.8)	6 (100)	249 (82.1)
Ischemic stroke	46 (51.7)*	22 (30.1)	40 (42.6)	3 (27.2)	2 (33.3)	100 (33.0)
Coronary heart disease	41 (46.1)	40 (54.8)	49 (52.1)	6 (54.5)	5 (83.3)	172 (56.8)
Diabetes	29 (32.6)	19 (26.0)	31 (33.0)	6 (54.5)	3 (50.0)	112 (37.0)

Footnote: *Comparison between groups by Chi-square test, the incidence of ischemic stroke was higher in current smokers than that in others (P < 0.01); **Comparison between groups by Chi-square test, Elder patients was more in never smoked than that in others (*P* < 0.05); ***Comparison between groups by One-Way ANOVA, Diastolic pressure in current smokers was higher than that in others (*P* < 0.05).

ferences among these groups were examined by x^2 test. Above tests were all two-tailed, *P* values less than 0.05 were considered statistically significant. SPSS17.0 version software package was applied during statistical analyses.

Results

576 participants (256 men and 320 women) aged from 60 to 95 years were enrolled (**Table 1**). The body mass index (BMI) of the elders showed normal distribution, and there was no significant difference between men and women in terms of BMI (*P* = 0.678). However, there were significant differences of such habits as smoking, drinking and exercising between men and women (*P* < 0.01), and the women education level was generally lower than men. Male current smokers accounted for 34.8% while female current smokers were only 3.4%. Biochemical tests showed that the incidence of HHcy was 38.0%, the prevalence of folate deficiency was 8.3%, hyperuricemia 18.0%, and low SOD 66.4%. Serum tHcy, UA, Folate and lipid indexes were significantly associated with gender (*P* < 0.001).

The survey found that current smokers were mainly the younger elders (*P* < 0.01), while the elders over 80 years old were almost never smokers (*P* < 0.05). Current smokers consumed 17 cigarettes per day, among which over 50% were "heavy smokers". Between never smokers and former smokers (**Table 2**), the diastolic blood pressure was significantly higher in male current smokers (χ^2 = 10.632, *P* = 0.005); and its proportion regarding to ischemic stroke was significantly higher than never smokers (χ^2 = 7.643, *P* = 0.022).

Table 3. The effect of cigarette smoking on biochemical indexes of participants

	Never smoker (n = 397)	Former smoker (n = 79)	Current smoker (n = 100)	F^1	P	F^2	P	P^3	P^4
Hcy** (umol/l)	14.11 ± 1.56	14.61 ± 1.52	16.83 ± 1.59	6.232	0.002	5.527	0.004	0.055	0.001
SOD* (U/l)	118.9 ± 21.7	123.0 ± 22.4	116.9 ± 23.5	1.758	0.173	5.464	0.004	0.013	0.002
CRP** (mg/l)	5.16 ± 2.45	7.92 ± 3.59	5.58 ± 2.54	6.011	0.003	4.471	0.012	0.297	0.055
Folate** (nmol/l)	22.54 ± 1.61	18.80 ± 1.53	15.58 ± 1.70	14.232	0.000	4.463	0.012	0.005	0.044
VB_{12}** (pmol/l)	347.2 ± 2.4	347.2 ± 1.6	287.1 ± 2.0	1.246	0.289	0.555	0.575	0.460	0.320
TC* (mmol/l)	4.47 ± 1.08	4.21 ± 1.16	4.40 ± 1.13	1.946	0.144	0.948	0.388	0.169	0.468
TG* (mmol/l)	1.53 ± 0.99	1.27 ± 0.59	1.59 ± 0.92	2.921	0.055	2.267	0.105	0.178	0.036
HDL* (mmol/l)	1.16 ± 0.32	1.05 ± 0.28	1.02 ± 0.23	11.105	0.000	2.027	0.133	0.045	0.287
LDL* (mmol/l)	2.53 ± 0.84	2.43 ± 0.91	2.61 ± 0.88	1.015	0.363	1.862	0.156	0.056	0.238
Glu** (mmol/l)	5.69 ± 1.33	5.91 ± 1.28	5.81 ± 1.34	0.687	0.503	0.661	0.517	0.972	0.310
HbA1c** (%)	6.62 ± 1.22	6.73 ± 1.28	6.75 ± 1.27	0.374	0.688	1.129	0.324	0.251	0.810
Cr** (μmol/l)	78.3 ± 1.5	91.8 ± 1.3	84.8 ± 1.3	7.829	0.000	0.332	0.718	0.536	0.443
UA** (μmol/l)	317.3 ± 1.4	327.0 ± 1.3	354.2 ± 1.3	5.056	0.007	4.779	0.009	0.036	0.003
Alb* (g/l)	37.8 ± 3.9	37.4 ± 4.3	37.8 ± 3.5	0.353	0.703	0.721	0.487	0.352	0.846
PA* (g/l)	0.25 ± 0.04	0.26 ± 0.04	0.26 ± 0.04	2.956	0.053	0.856	0.425	0.538	0.498

Footnotes: * normal distribution variables described as mean ± standard deviation; ** The asymmetric distribution of the variables described as Geometric mean ± standard deviation after natural logarithm transformation; F^1, Compared differences between groups by One-Way ANOVA; F^2, The general linear model was used to compared the differences between groups, All variables after adjustment for age, gender, BMI, hypertension and diabetes; UA, Uric acid, after adjustment for serum creatinine; SOD after adjustment for serum albumin; Hcy, after adjustment for serum creatinine, Folate and VB_{12}; P^3, Pair-wise comparison between current smokers and never smokers; P^4, Pair-wise comparison between current smokers and former smokers.

Effects of smoking on biochemical indexes

The comparisons of biochemical indexes among current smokers, never smokers and former smokers were shown in **Table 3**. One-way analysis of variances showed that serum tHcy, UA concentrations tended to increase in current smokers, while folate showed a downward trend, and these differences were statistically significant (P = 0.002, P = 0.007, P = 0.000); after correcting the confounding factors by the linear model, the results were similar to the former. Pairwise comparison came to the results that the difference between current smokers and never smokers or former smokers was statistically significant. Variance analysis also showed that serum HDL in current smokers decreased (P = 0.000), while Cr increased (P = 0.000). However, after the adjustment for confounding factors, the results showed no statistical significance (P = 0.133, P = 0.718). On the contrary, one-way analysis of variances showed that SOD in the three groups had no significant difference. But after correcting confounding factors, SOD amount of current smokers was significantly lower than those in never smokers (P = 0.013) and former smokers (P = 0.002). In addition, compared with never smokers and former smokers, the levels of TC, TG, GLU, HbA1c, Alb and PA in current smokers had no statistically significant differences.

Relationship between smoking consumption and biochemical indexes

As shown in **Table 4**, after correcting such confounding factors as age, sex and blood pressure, univariate analyses revealed that serum tHcy was positively associated with daily smoking consumption (P = 0.020) and the pack-years of smoking (P = 0.003); serum SOD was negatively correlated with daily smoking consumption (P = 0.103) and pack-years of smoking (P = 0.061), without statistically significant. There was no related statistically significance between daily smoking consumption and smoking related indexes in terms of serum folate and UA (P > 0.05).

The results of multivariate logistic regression analysis showed that current smoking status was an independent risk factor of increase of serum tHcy (β = 0.985, OR = 2.677,95% CI: 1.182-6.065, P = 0.018) as well as rise of UA (β = 0.920, OR = 2.510,95% CI: 1.194-5.274, P = 0.015), apart from age, serum folate, VB_{12} and Cr. Moreover, except from age, smoking, albumin, the rise of tHcy level (β = 1.244, OR = 3.469, 95% CI: 2.148-5.601, P = 0.000) was

Table 4. Relationship between the number of cigarette smoked and biochemical indexes in current smokers

	Cigarette per day		Pack-years of smoking	
	r	*p*	*r*	*p*
Hcy*		0.020	0.386	0.003
SOD**	-0.181	0.103	-0.208	0.061
Folate**	-0.067	0.619	-0.103	0.442
UA**	0.007	0.947	0.034	0.752

Footnotes: *Adjustment for age, gender, blood pressure, body mass index, serum folate, vitamin B_{12}, albumin and Creatinine; **Adjustment for age, gender, blood pressure, body mass index, serum albumin and Creatinine.

an independent risk factor of the decrease of SOD level.

Relationship between smoking-related biochemical indexes and cardiovascular disease

The data of smoking-related biochemical indexes, serum UA, tHcy, SOD and folate, were described as quintile, and the relationships of cardiovascular diseases with the increase of serum UA, tHcy and with the decrease of SOD, folate were analyzed (**Table 5**). After adjusting multiple confounding factors, elevated UA increased the risk of coronary heart disease (OR = 2.478, 95% CI: 1.173-5.237, *P* = 0.017) and reduced the risk of ischemic stroke (OR = 0.280, 95% CI: 0.141-0.557, *P* = 0.000). The decreasing of serum SOD increased the risk of hypertension (OR = 2.748, 95% CI: 1.151-6.561, *P* = 0.023), coronary heart disease (OR = 2.343, 95% CI: 1.155-4.752, *P* = 0.018) and ischemic stroke (OR = 2.168, 95% CI: 1.056-4.453, *P* = 0.035); however, the increasing of tHcy did not increase the risk of hypertension (OR = 0.816, 95% CI: 0.320-2.078, *P* = 0.670) or coronary heart disease (OR = 0.980, 95% CI: 0.453-2.121, *P* = 0.959), but may increase the risk of ischemic stroke (OR = 1.191, 95% CI: 0.936-3.932, *P* = 0.075).

Discussion

Taking geriatric cardiovascular patients as the participants, we analyzed the aspect of smoking habit for the first time in our study. Among the current smokers, the women accounted for about 10%. Current smoking and cessation rates for males were 34.8% and 28.5% respectively, which were higher than that Saw et al. [13] had reported about those Chinese middle-aged males living in Singapore (31% and 25% respectively). The average pack-years of smoking in current smokers reached 35, in which men had a daily average smoking of 18 cigarettes (53% for heavy smokers) and women had a daily average smoking of 15 cigarettes (27% for heavy smokers). The numbers of heavy smokers both in men and women were more than that reported in a survey about healthy people of a similar age [14]. The present study indicated that smoking was a risk factor for elevated diastolic blood pressure and strokes in males. In addition, the males never smoked over 80 years old, accounted for a markedly increasing proportion, from which it can be inferred that smoking will do harm to physical health, increase the risk of cardiovascular diseases and influence the life expectancy.

In our study, the tHcy level of the enrolled patients was positively correlated with age. The geometric mean of tHcy in males and females were 16.07 ± 1.60 umol/l and 13.54 ± 1.52 umol/l, respectively, which were both higher than those in aged healthy population in America (11.91 ± 0.19 umol/l, 11.30 ± 0.41 umol/l) [15, 16], and the Singapore Chinese reported by Seang-Mei Saw [12.8 (11.8-13.9) umol/l, 10.3 (9.5-11.0) umol/l]. Taken tHcy over 15 umol/l as a judging criterion of HHcy, the incidence of HHcy was 44.5% for males and 35.3% for females. Since McCully raised the mechanism of tHcy in atherosclerosis for the first time in 1969, numerous epidemiologic studies has indicated that elevated tHcy level was associated with the increased risk of atherosclerosis in heart and cerebral vessels and vessels of limbs, and was also an independent risk factor of stroke in elder people [17, 18]. Bots et al. found that the stroke risk would increase with 6% to 7% for each increase of 1 μmol/l in tHcy level, through a comparison study on the geriatric patients [19]. However, recent population intervention studies have found that the supplement of folic acid and VB_{12} could reduce the Hcy level but not decrease the risk of cardiovascular events [20]. In present study, by adjusting various confounding factors, we found that the elevated tHcy level in elderly patients did not raise the risk of hypertension or coronary heart disease, but may increase the risk of ischemic stroke.

Our survey found that the serum tHcy level in current smokers had a rising trend. To investigate how smoking affects the Hcy level, the

Table 5. The relationship between increased or decreased* biochemical indexes related smoking and the risk of cardiovascular disease

	Hypertension		Coronary Heart Disease		Ischemic Stroke	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Uric acid level						
Age-, sex-adjusted	1.589 (0.821-3.078)	0.170	3.161 (1.771-5.643)	0.000	0.425 (0.232-0.781)	0.006
Multivariate-adjusted**	0.938 (0.368-2.390)	0.894	2.478 (1.173-5.237)	0.017	0.280 (0.141-0.557)	0.000
SOD level						
Age-, sex-adjusted	0.884 (0.425-1.839)	0.742	2.344 (1.283-4.283)	0.006	0.827 (0.446-1.531)	0.545
Multivariate-adjusted**	2.748 (1.151-6.561)	0.023	2.343 (1.155-4.752)	0.018	2.168 (1.056-4.453)	0.035
Homocysteine level						
Age-, sex-adjusted	1.478 (0.725-3.014)	0.283	1.626 (0.901-2.934)	0.106	1.372 (0.750-2.512)	0.305
Multivariate-adjusted2	0.816 (0.320-2.078)	0.670	0.980 (0.453-2.121)	0.959	1.919 (0.936-3.932)	0.075
Folate level						
Age-, sex-adjusted	0.648 (0.216-1.944)	0.439	0.809 (0.335-1.958)	0.639	1.937 (0.799-4.697)	0.143
Multivariate-adjusted**	0.650 (0.168-2.507)	0.532	0.788 (0.293-2.122)	0.638	1.736 (0.704-4.281)	0.231

Footnotes: *UA and Hcy, the highest quintile compared with the lowest quintile; SOD and Folate, the lowest quintile compared with the highest quintile; **Adjusted for age, sex, diabetic status, body mass index, systolic blood pressure, serum total cholesterol, serum creatinine, C reactive protein, leisure time physical activity, drinking, and use of aspirin in the past month.

influence of smoking on the serum folate and VB_{12} were analyzed. The results showed that the serum folate level in former smokers and current smokers were both lower than that in the never smokers, and the lowest level was of current smokers. Folic acid plays a critical role in the synthesis of DNA, methylation and cell repair, and is the major methyl donor in the methylation of Hcy together with VB_{12}. Lots of investigations have confirmed that the serum folate is negatively correlated with the serum tHcy level [21, 22]. And smoking was independently associated with the elevated tHcy level in this study. The present results combining with previous studies [23, 24] indicated that the mechanism of the above changes caused by smoking might be account for the blocked pathway of Hcy methylation into methionine. The methyl donor is used to repair cell damage from oxidation produced by tobacco. Therefore, we believed that the elevated tHcy level caused by smoking is partly associated with the decreased of folic acid.

Our findings also indicated there were about 66% of the enrolled patients had low serum SOD levels associated with various factors such as age, gender and level of albumin. After adjustment for the confounding factors like age, gender and diseases, the serum SOD level in current smokers was found to be lower than those of both never smokers and former smokers. This is in conformity with the previous study from Wang et al. In Wang's study, the serum SOD level in current smokers was lower than both former smokers and never smokers with former smokers at the in-between level [25]. The tobacco smoke contains a large amount of free radicals which could enter into the systemic circulation and directly act on the entire vascular bed to induce the oxidative stress. Active oxidants in the tobacco smoke are one of the critical causes of the damage to protein, lipid and DNA [26, 27]. The elimination of peroxide firstly depends on the intracellular and extracellular SOD, which would involve in the disproportionate reaction to get rid of the free radicals in case of excessive superoxide anion. When smoking, the body will compel anti-oxidant to eliminate excessive free radicals, causing the change of antioxidant system [28]. The present results confirmed the oxidation mechanism how tobacco damaged the vessels. Multivariate logistic regression analysis indicated that the elevated serum tHcy level was independently associated with the reduced serum SOD level. In addition, the present study also found that decreased serum SOD level increased the risk of cardiovascular diseases while the elevated tHcy level not. Consequently it can be concluded the elevated tHcy level may be associated with the change in metabolism caused by the impaired anti-oxidation defense system.

Then, a further analysis about the influence of daily smoking consumption and the pack-years of smoking on the serum biochemical indicators was performed, which showed that the daily and annual smoking quantity was positively correlated with serum tHcy concentrations, and negatively correlated with serum SOD levels. Accordingly, we made an inference that smoking activated the systemic oxidation

reaction system and damaged the anti-oxidation defense system. When smoking, the consumption of the methyl donors used to repair cell damage exists continuously and will increase with the elevating smoking quantity, which in turn continuously block the methylation of Hcy and result in the accumulation of Hcy. Smoking can result in the reduction of bioavailability of nitric oxide [5] and the oxidative stress is recognized as a common pathological factor of the endothelial cell dysfunction and the development and progression of atherosclerosis. Stein et al. conducted some investigations and found that young smokers may have a reduced tHcy level if choosing to stop smoking, while the decrease in smoking quantity would not alter the tHcy level [29]. Stein also believed that the diminishment of smoking quantity would not alleviate the smoking associated vascular damage. However, our study found that there was a dose-dependent relationship between the serum tHcy level and smoking quantity. Increase in tHcy level was proportional to the smoking quantity or the exposure to smoking, which indicated that smoking associated vascular damage might aggravate continuously. As a result, both reducing smoking quantity and stopping smoking at any time will benefit for elderly patients enrolled in this study.

Conclusion

Smoking is still common in elderly patients with cardiovascular disease, which elevates the diastolic blood pressure in males, increases the risk of stroke and affects the life expectancy. It is not only associated with the reduced serum folic acid, but also the elevated serum tHcy. What's more, the daily smoking consumption and the pack-years of smoking directly influence the serum tHcy level. Smoking is associated with the reduced serum SOD, which increases the risk of hypertension, coronary heart disease and stroke. The elevated Hcy level in cardiovascular patients is probably associated with changes in metabolism after damage of the body from oxidation.

This study is a cross-sectional study which lacks enough evidences to explain the relationship between cardiovascular diseases and biochemical indexes and the exposure of risk factors. This study mainly focuses on Chinese elderly, with a single ethnic population, so there are Chinese characteristics in the daily habits of eating, drinking and smoking, etc. Although we have taken the effects of confounding factors into consideration in the statistical analysis, coexist diseases, drugs and environmental changes could interference the data due to that the participants of this study are hospitalized patients.

Disclosure of conflict of interest

None.

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