Letter to the Editor

High Prevalence and Factors Contributing to Hyperhomocysteinemia, Folate Deficiency, and Vitamin B_{12} Deficiency among Healthy Adults in Shanghai, China

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Elevated plasma or serum total homocysteine (tHcy) level has been established as a risk factor for cardiovascular disease[1], as well as dementia and cognitive decline[2]. Plasma or serum folate and vitamin B_{12} influence homocysteine (Hcy) metabolism as a co-substrate and cofactor respectively, so that low concentrations of folate and vitamin B_{12} are also associated with high Hcy levels[1]. However, not much information is available describing serum tHcy, folate, and vitamin B_{12} status in Shanghai adults, especially in a healthy population. Therefore, we hypothesize that low serum folate and vitamin B_{12} is associated with high Hcy in healthy adults in Shanghai. The aim of this study was to determine the status of serum tHcy, folate, and vitamin B_{12}, and the prevalence and factors contributing to HHcy, folate deficiency, and vitamin B_{12} deficiency among healthy adults in Shanghai, China.

Data of this study were obtained from 'Surveillance of Chronic Non-communicable Diseases in Shanghai, 2013'. A stratified multistage cluster sampling was conducted. Sixty urban townships, 30 suburban townships, and 30 rural townships were randomly sampled out for each type, separately. In the second stage, 4 villages or residential areas were sampled from each selected township by using the method of probability proportion to size. In the following sampling stages, 2 residential groups (at least 50 families) were sampled out from each village by using the simple random sampling method, and then a family member aged 15 years and above from each family was selected using the Kish grid method. The sample size was 25,657. Those who were 35 years old and above, and did not meet the exclusion criteria were selected as the subjects of this study. The exclusion criteria were: 1) Subjects who had been diagnosed with hypertension or whose systolic blood pressure (SBP) and/or diastolic blood pressure (DBP) were higher than 140 and/or 90 mmHg, respectively; 2) Subjects who had been diagnosed with diabetes and whose fasting blood glucose level was higher than 7.0 mmol/L; and 3) Subjects who had been diagnosed with cardiovascular disease or cancer. Finally, 8,337 subjects were included.

The questionnaire was obtained from the World Health Organization global non-communicable disease integrated monitoring framework (25 indicators) and was inquired by trained investigators. Height was measured with a stadiometer of 2 meters and an accuracy of 0.1 centimeters. Weight was measured with a weighing scale allowing up to a maximum of 150 kilograms and an accuracy of 0.1 kilograms. Blood pressure was measured using OMRON HEM-7071 electronic sphygmomanometer (Omron Co., Ltd., Dalian, China). Fasting blood samples were obtained after 8 hours of overnight fasting. Cold transportation and all measurements of blood samples were uniformly performed by ADICON Clinical Laboratory Inc., an independent chain clinical reference laboratory in China. Serum tHcy was determined using enzymatic cycling on a Beckman Coulter AU680 analyzer. Serum folate and vitamin B_{12} concentrations were analyzed using Roche imported reagents and E601 with an automatic electric luminescence analyzer.

According to the 'Chinese Guidelines on Prevention and Treatment of Dyslipidemia in Adults', high levels of total cholesterol (TC) was defined as serum cholesterol ≥ 6.22 mmol/L; high levels of low-density lipoprotein cholesterol (LDL-C) was defined as serum LDL-c ≥ 4.14 mmol/L; low levels of high-density lipoprotein cholesterol (HDL-C) was defined as serum HDL-C < 1.04 mmol/L; and high levels of triglycerides (TG) was defined as serum TG ≥ 2.26 mmol/L. Abnormal creatinine (Cr) level was defined as serum Cr > 110 μmol/L for men and > 96 μmol/L for women, and high levels of high-sensitivity C-reactive protein (hs-CRP) was defined as serum hs-CRP > 3.0 mg/L according to the reagent

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specifications. HHcy was defined as serum Hcy > 15 μmol/L[3]. Folate deficiency was defined as serum folate < 10.4 nmol/L and vitamin B_{12} deficiency was defined as serum vitamin B_{12} < 156 pmol/L according to the reagent specifications. Current smoking was defined as smoking during the survey. Drinking was defined as drinking any of various alcoholic beverages purchased or made.

Means and standard deviations were used to describe continuous variables. Due to the skewed distribution, the geometric mean (GM) was used to describe the mean levels of serum tHcy, folate and vitamin B_{12}. The *t*-test and Pearson's chi-square test were used to compare differences regarding gender, age and area. The factors contributing to HHcy, folate deficiency, and vitamin B_{12} deficiency were determined by logistic regression analyses (stepwise method, with the occurrence of HHcy/folate deficiency/vitamin B_{12} deficiency as a dependent variable; gender, age, area, body mass index (BMI), SBP, DBP, high TC, high LDL-C, low HDL-C, high TG, hs-CRP, Cr, HHcy, folate deficiency, and vitamin B_{12} deficiency were analyzed as independent variables; included standard 0.05, excluded standard 0.10; α = 0.05). Data were analyzed using SPSS version 20 (IBM Corp., Armonk, NY, USA).

The mean age, BMI, SBP, DBP, TG, hs-CRP, and Cr were higher while TC, LDL-C, and HDL-C were lower in male subjects than in female subjects. A significantly higher proportion of heavy drinking frequency and current smoking were found among male versus female subjects (Table 1).

Table 1. Characteristics of Subjects in this Survey

Characteristics	Men (*n* = 3,100)	Women (*n* = 5,237)	Total (*n* = 8,337)
Area, *n* (%)*			
Urban	1,438 (46.4)	2,732 (52.2)	4,170 (50.0)
Suburb	753 (24.3)	1,267 (24.2)	2,020 (24.2)
Rural	909 (29.3)	1,238 (23.6)	2,147 (25.8)
Age (years), *n* (%)*	56.3 ± 11.4	55.4 ± 10.8	55.7 ± 11.1
35-44	545 (17.6)	885 (16.9)	1,430 (17.2)
45-59	1,376 (44.3)	2,663 (50.8)	4,039 (48.4)
60-74	972 (31.4)	1,403 (26.8)	2,375 (28.5)
≥ 75	207 (6.7)	286 (5.5)	493 (5.9)
BMI (kg/m^2)*	23.6 ± 3.0	23.4 ± 3.0	23.4 ± 3.0
SBP (mmHg)*	123.4 ± 9.8	120.6 ± 11.0	121.7 ± 10.7
DBP (mmHg)*	77.3 ± 6.8	75.8 ± 7.1	76.3 ± 7.0
TC (mmol/L)*	5.3 ± 1.1	5.6 ± 1.1	5.5 ± 1.1
LDL-c (mmol/L)*	2.7 ± 0.7	2.9 ± 0.7	2.8 ± 0.7
HDL-C (mmol/L)*	1.3 ± 0.3	1.5 ± 0.3	1.4 ± 0.3
TG (mmol/L)*	1.6 ± 1.2	1.5 ± 1.2	1.5 ± 1.2
FBG (mmol/L)#	5.3 ± 0.6	5.3 ± 0.6	5.3 ± 0.6
hs-CRP*	1.8 ± 4.4	1.4 ± 3.2	1.7 ± 3.8
Cr mmol/L*	86.3 ± 21.1	71.2 ± 9.6	76.8 ± 16.6
Current smoking, *n* (%)*	2,976 (64.2)	112 (1.6)	3,088 (26.4)
Drinking frequency, *n* (%)*			
Never/< 1/Mo	1,736 (56.0)	4,851 (92.7)	6,587 (79.0)
1-3/Mo	237 (7.6)	142 (2.7)	379 (4.5)
1-4/Wk	411 (13.3)	122 (2.3)	533 (6.4)
5-7/Wk	716 (23.1)	122 (2.3)	838 (10.1)

Note. *P < 0.001; #P < 0.05. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides; FBG: fasting blood glucose; hs-CRP: high-sensitivity C-reactive protein; Cr: creatinine.

The GMs of serum tHcy, folate and vitamin B_{12} level were 12.7 [95% confidence interval (*CI*): 12.6-12.8] μmol/L, 21.4 (95% *CI*: 21.2-12.6) nmol/L and 376.5 (95% *CI*: 373.3-379.7) pmol/L, while the prevalence rates of HHcy, folate deficiency and vitamin B_{12} deficiency were 24.7% (95% *CI*: 23.7%-25.6%), 4.7% (95% *CI*: 4.3%-5.2%) and 1.9% (95% *CI*: 1.6%-2.2%), respectively. Males had significantly higher prevalence rates of HHcy, folate deficiency and vitamin B_{12} deficiency compared with females. Subjects aged 75 years and older had significantly higher prevalence rates of HHcy, folate deficiency and vitamin B_{12} deficiency than other age groups. Subjects living in the suburbs had a relatively higher prevalence of HHcy than those living in urban or rural areas (Table 2).

Adjusting for age and gender, multivariate analysis revealed that HHcy was positively associated with SBP, low levels of HDL-C, high levels of hs-CRP, abnormal Cr, folate deficiency, vitamin B_{12} deficiency, and smoking, and negatively associated with alcohol drinking frequency. Folate deficiency was positively associated with low levels of HDL-C, high levels of hs-CRP, and smoking and negatively associated with overweight. Vitamin B_{12} deficiency was positively associated with low levels of HDL-C and negatively associated with high levels of TC and abnormal Cr levels. Subjects with folate deficiency and vitamin B_{12} deficiency were more likely to have HHcy (*OR* = 11.300 and 4.544, respectively) (Table 3).

Elevated tHcy concentration is an independent risk factor for cardiovascular diseases[1]. Stratified analyses based on Chinese geographical area showed that the prevalence of HHcy was high in the northern areas (34.3%), intermediate in the central areas (21.0%), and low in the southern areas (16.0%). Inlanders (31.5%) had a higher prevalence of HHcy than those living in coastal areas (23.0%)[3]. The pooled result is consistent with our study result (24.7%) since Shanghai is an intermediate and coastal city in China.

Serum tHcy levels and prevalence of HHcy among males or older subjects were higher than those of females or younger subjects observed in this study, which was consistent with observations from a published study[4]. Several possible reasons for the high tHcy level in elderly subjects have been given; changes in renal function and impaired renal metabolism of Hcy might be involved[5]. Our study also found that the mean serum Cr level was positively associated with the risk of HHcy.

Table 2. Statuses of HHcy, Folate Deficiency and Vitamin B_{12} Deficiency

Items	Serum tHcy (μmol/L)		HHcy		Serum Folate (nmol/L)		Folate Deficiency		Serum Vitamin B_{12} (pmol/L)		Vitamin B_{12} Deficiency	
	GM	95% *CI*	%	95% *CI*	GM	95% *CI*	%	95% *CI*	GM	95% *CI*	%	95% *CI*
Total	12.7	12.6-12.8	24.7	23.7-25.6	21.4	21.2-21.6	4.7	4.3-5.2	376.5	373.3-379.7	1.9	1.6-2.2
Gender												
Men	15.1	14.9-15.3	42.3	40.8-43.7	18.1	17.8-18.3	9.8	9.0-10.5	342.3	337.5-347.2	2.7	2.2-3.2
Women	11.5	11.4-11.6	14.2	13.1-15.3	23.7	23.5-23.9	1.7	1.1-2.3	398.3	394.1-402.5	1.4	1.0-1.8
P-value	< 0.001		< 0.001		< 0.001		< 0.001		< 0.001		< 0.001	
Age (years)												
35-44	11.9	11.7-12.2	20.3	18.1-22.5	20.4	20.0-20.8	5.2	4.1-6.3	376.1	368.7-383.7	1.7	1.0-2.4
45-59	12.2	12.1-12.3	19.7	18.4-21.0	21.9	21.6-22.2	4.0	3.4-4.7	388.0	383.4-392.6	1.2	0.8-1.6
60-74	13.5	13.3-13.7	30.9	29.2-32.7	21.5	21.2-21.9	4.8	3.9-5.6	366.8	360.8-372.9	2.4	1.9-3.0
≥ 75	15.6	15.0-16.2	47.7	43.9-51.4	20.3	19.5-21.0	8.5	6.7-10.4	334.1	320.2-348.5	5.3	4.1-6.5
P-value	< 0.001		< 0.001		< 0.001		< 0.001		< 0.001		< 0.001	
Area												
Urban	12.6	12.5-12.7	23.6	22.3-25.0	21.6	21.3-21.8	4.2	3.5-4.8	386.1	381.4-390.9	1.8	1.4-2.2
Suburb	12.9	12.7-13.1	26.6	24.7-28.5	20.8	20.5-21.2	5.0	4.1-6.0	360.5	354.4-366.7	2.1	1.5-2.7
Rural	12.7	12.5-12.9	24.8	23.0-26.6	21.8	21.4-22.1	5.4	4.5-6.3	373.3	367.2-379.5	1.7	1.2-2.3
P-value	0.015		0.041		0.001		0.064		< 0.001		0.595	

Our study found that HHcy was significantly associated with deficiency of folate and vitamin B_{12}. We also found that there were strongly moderate negative correlations between Hcy and folic acid (R = -0.666, P < 0.001) and vitamin B_{12} (R = -0.564, P < 0.001)[6]. In our study, the subjects aged 75 years and older had lower GMs of serum folate and vitamin B_{12} compared with subjects aged 35-44 years, which might have caused the high prevalence of HHcy among elderly subjects. Elevated plasma Hcy concentration in older populations could also be attributed to malabsorption of vitamin B_{12} by the aging gut[7]. This study showed that overweight was negatively associated with folate deficiency, and high TC was negatively associated with vitamin B_{12} deficiency, which means overweight and high TC would be protective factors for HHcy, indirectly.

Our study showed that SBP was positively associated with the risk of HHcy. Previous studies showed that SBP was an important determinant of microalbuminuria and the probability of having stage 1 and stage 2 chronic kidney disease was significantly higher in subjects with greater values of SBP[8]. Impaired renal metabolism of Hcy may be caused by raising the SBP.

Our results showed that low HDL-C was associated with not only HHcy but also folate deficiency and vitamin B_{12} deficiency. It was reported that Hcy reduced the concentration of HDL-C in plasma by inhibiting the hepatic synthesis of apolipoprotein A1 (apoA-I), the main HDL apolipoprotein. An Hcy-induced inhibition of apoA-I synthesis was the mechanism linking Hcy to the development of atherosclerosis[9]. Prior epidemiologic evidence had positively linked plasma measures of folate with HDL-C[10] and apoA-I[11] levels.

CRP is a marker of systemic inflammation that has been associated with an increased risk of myocardial infarction and stroke. CRP has also been confirmed in the study to be positively associated with the level of Hcy[12]. Our results confirmed that elevated serum hs-CRP is an independent risk factor for HHcy and folate deficiency.

A study suggested that cigarette smoking was a strong determinant of plasma tHcy level[13]. Our result showed that current smoking was positively associated with the prevalence of HHcy and folate deficiency, which was consistent with the published data[13]. That also partly attributed the high GM of serum level of tHcy and high prevalence of HHcy among male subjects to the high current smoking rate compared with female subjects caused by the social custom in our study.

Our study found that there was a protective effect for HHcy among individuals who had the highest drinking frequency compared to those who had never or seldom drank, which might be related to

Table 3. Factors Contributing to HHcy, Folate Deficiency and Vitamin B_{12} Deficiency

Variables	HHcy			Folate Deficiency			Vitamin B_{12} Deficiency		
	OR	95% *CI*	*P*	*OR*	95% *CI*	*P*	*OR*	95% *CI*	*P*
BMI = 24.0-27.9 kg/m^2 (*vs.* < 24)	0.979	0.862-1.111	0.738	0.758	0.595-0.966	0.025	0.957	0.663-1.383	0.816
SBP	1.009	1.002-1.017	0.010	0.995	0.982-1.008	0.449	1.011	0.991-1.032	0.291
High TC (*vs.* normal TC)	0.878	0.755-1.021	0.091	1.092	0.812-1.469	0.560	0.548	0.324-0.928	0.025
Low HDL-C (*vs.* normal HDL-C)	1.242	1.037-1.487	0.019	1.536	1.158-2.037	0.003	1.656	1.062-2.583	0.026
High hs-CRP (*vs.* normal hs-CRP)	1.021	1.006-1.036	0.007	1.024	1.006-1.042	0.010	0.997	0.965-1.030	0.838
Abnormal Cr (*vs.* normal Cr)	1.060	1.054-1.066	< 0.001	1.002	0.998-1.006	0.450	0.969	0.954-0.984	< 0.001
HHcy (*vs.* normal tHcy)	-	-	-	5.103	3.995-6.518	< 0.001	11.470	7.560-17.420	< 0.001
Folate deficiency (*vs.* normal folate)	4.544	3.546-5.824	< 0.001	-	-	-	1.499	0.913-2.461	0.110
Vitamin B_{12} deficiency (*vs.* normal vitamin B_{12})	11.300	7.420-17.210	< 0.001	1.538	0.939-2.521	0.088	-	-	-
Smoking (*vs.* no smoking)	1.460	1.236-1.724	< 0.001	1.961	1.463-2.627	< 0.001	0.788	0.498-1.248	0.310
Drinking 5-7/wk (*vs.* never drinking)	0.767	0.633-0.929	< 0.001	0.925	0.676-1.267	0.627	1.199	0.701-2.051	0.507

Note. *OR*s and 95% *CI*s were adjusted for the demographic factors of gender and age in the logistic regression model.

the activation of the betaine Hcy methyltransferase pathway which had become more important as a source of S-adenosylmethionine and a determinant of Hcy in alcohol consumers due to the inhibition of methionine synthase[14]. However, further research is needed in this regard.

In conclusion, about a quarter of healthy adults had HHcy in Shanghai. HHcy can be influenced directly by folate and vitamin B_{12} level, SBP, serum lipid, Cr, hs-CRP, smoking, and alcohol drinking frequency, as well as indirectly by overweight. Recommended intakes of folate and vitamin B_{12} supplements can reduce serum Hcy levels, which may help in preventing cardiovascular diseases and dementia.

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