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The Relationship between Risk Factors for Cardiovascular Disease and Levels of Plasma Total Homocysteine, Folate and Vitamin B_{12} in Koreans

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Abstract

The elevation of total plasma homocysteine is now an established risk factor for cardiovascular disease. Plasma folate and vitamin B_{12} influence Hcy metabolism as cofactors. In this study, we studied the relationship of major risk factors for cardiovascular disease, including advanced age, male gender, obesity, hypertension, hyperglycemia, and dislipidemia and plasma homocyteine, folate and vitamin B_{12} levels in Koreans. A total of 195 adult Koreans participated. The subjects were divided into three groups according to how many major conventional risk factors of cardiovascular disease they had: no risk, low risk (1~3 risk factors) and high risk (> 3 risk factors) groups. As the number of risk factors increased, the plasma homocysteine levels significantly increased, while the plasma folate levels significantly decreased. The plasma homocysteine levels were higher in males than in females. The subjects with hyperglycemia had higher plasma homocysteine levels than the subjects without the risk factor. Also the subjects with dislipidemia had higher plasma homocysteine levels than the subjects without the risk factor. The plasma folate and vitamin B_{12} levels were significantly lower in males than females. However, there were no significant differences in plasma folate and vitamin B_{12} levels between the subjects with or without other risk factors. These results indicate that plasma homocysteine levels were positively related with risk factors for cardiovascular disease and plasma folate levels were negatively related with the risk factors for cardiovascular disease. Also, we conclude that plasma homocysteine levels might be related to the combination of risk factors, rather than an individual risk factor.

Key words: homocysteine, folate, vitamin B_{12}, cardiovascular disease

INTRODUCTION

The risk factors for the development of cardiovascular disease, which is the main cause of mortality in Korea, are associated with advanced age, male gender, obesity, hypertension, hyperglycemia, and dislipidemia like as in Western countries (1). Recently, elevated total homocysteine levels have been identified as an independent risk factor for cardiovascular disease (2-5). Although the mechanisms of hyperhomocysteinemia leading to cardiovascular disease, including atherosclerosis are still not completely elucidated (6), some investigators have shown that hyperhomocysteinemia can cause vascular endothelial dysfunction both *in vitro* (7-9) and *in vivo* (10,11). Also, in epidemiologic studies, homocysteine levels above the 80th percentile of the normal range have been reported in almost 40% of the patients with vascular disease, including coronary heart disease (12). Meta-analysis of 17 studies involving 5230 individuals suggests that a 1 μmol/L increment in total homocysyteine levels is associated with a 10% increase in coronary heart disease risk (13). Furthermore, they reported that a 5 μmol/L increase in total homocysteine levels was associated with an odds ratio (OR) of 1.6 among men and 1.8 among women (13).

The homocysteine levels are modulated by the interaction between a number of nutritional and genetic factors including the B vitamins, folate, vitamin B_{12}, and vitamin B_6 (14). Data from the Framingham Heart Study indicate that two-thirds of elevated plasma homocysteine levels may be secondary to moderate or low levels of vitamin B_6, vitamin B_{12}, and folate (13). Kang et al. (15) reported that 56% of people with low to normal folate levels have elevated plasma homocysteine levels. Therefore, it is assumed that plasma B vitamin status are negatively related to the risks of cardiovascular disease while plasma homocysteine levels are positively related to the risks of cardiovascular disease. However, data on the distribution of plasma homocysteine levels in Korean are lacking and only limited information is available describing the correlations of elevated plasma homocysteine to cardiovascular disease.

It is important to evaluate the relation of homocysteine with each risk factor for cardiovascular disease, because mechanisms of cardiovascular disease are multifactorial, and these risk factors are clustered together (16,17). In this study, we investigated the relationship of plasma homocysteine, folate and vitamin B_{12} levels to the risk factors for cardiovascular disease in Koreans.

MATERIALS AND METHODS

Subjects

A total of 195 healthy volunteers (99 males, 96 females)

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who did not knowingly suffer from any disease, and who were not receiving any medical treatment participated.

Methods

Height, weight, and systolic (SBP) and diastolic (DBP) blood pressure of all individuals were measured. Samples for plasma were taken in the fasting state (overnight). Plasma levels of total cholesterol, triglyceride, highdensity lipoprotein (HDL)-cholesterol, and glucose were analyzed by enzymatic methods using commercial kits (Eiken Co., Japan). Plasma levels of homocysteine were determined by using high pressure liquid chromatography with a fluosencence detector according to the modified methods of Araki et al. (18) and Refsum et al. (19). Plasma was treated before it was applied to the analytical column : reduction with tri-n-butylphosphine, protein precipitation, and derivatization with 7-fluoro-benzo-2-oxa-1,3-diazole-4-sulfonate. Thiol adducts were separated by using an isocratic system with a 0.1 M acetic acid/acetate buffer (pH 5.5, containing 3% methanol) as a mobile phase and detected by fluorescence (excitation at 385 nm, emission at 515 nm). The flow rate was 0.7 mL/min and cysteamine was used as an internal standard. Plasma levels of folate were measured by using the *Lactobacillus casei* microbiological assay (*L. casei* ATCC 7469) (20). Plasma vitamin B_{12} levels were analyzed by using a radiobinding assay kit (Chiron Diagnostics Co., Norwood, MA, USA).

Major risk factors of cardiovascular disease including advanced age, male gender, obesity, hypertension, hyperglycemia, and dislipidemia were screened in each patient. Advanced age was defined as age >45 years old in men and >55 years old in women. Obesity was defined as BMI≥25. Hypertension was defined as SBP/ or DBP≥160/95 mmHg. Hyperglycemia was defined as a fasting plasma glucose concentration≥140 mg/dL. Dislipidemia was defined as the case in which subjects had one or more of the following abnormal lipid levels: hypercholesterolemia (≥240 mg/dL), hypertriglyceriemia (≥250 mg/dL), high LDL-cholesterol (≥160 mg/dL), or low HDL cholesterol (<35 mg/dL), respectively.

These 195 subjects were divided into three groups according to how many major conventional coronary risk factors they had: no, low, and high risk groups. The no risk group consisted of 48 subjects who had no risk factors, the low risk group consisted of 115 subjects who had 1-3 risk factors, and the high risk group consisted of 32 subjects who had <3 risk factors.

Statistical analysis

Data analysis was performed with SAS Statistical Software (SAS Institute, Cary, NC). Values are expressed as mean and standard deviation for continuous variables and as percentage and number for categorical variables. General linear model (GLM) with Duncan's Multiple range test was used to assess the differences of mean levels of continuous variable among the groups. Student's t test was used to compare the plasma homocysteine levels between subjects with or without major risk factors. Relations between risk factor and plasma homocysteine, folate and vitamin B_{12} levels were calculated using Pearson's correlation coefficient.

RESULTS

Clinical and biochemical characteristics

The characteristics of the subjects are summarized in Table 1. One hundred and ninety five adults (male: 99, female: 96) participated. The distribution of age was 37 (m: 17, f: 20) for 20-year olds, 47 (m: 30, f 17) for 30-year old, 43 (m: 24, f: 19) for 40-year old, 40 (m: 15, f: 25) for 50-year old, and 28 (m: 13, f: 15) for above 60-year old. The mean values of age and BMI were 43.9±3.8 years and 22.9±3.1 kg/m^2, respectively. The mean levels of blood pressure (SBP/DBP) and fasting glucose were 121.4±13.2 and 73.6±9.7 mmHg and 93.1±33.0 mg/dL. The mean plasma lipid levels were 108.5±75.4 for triglyceride, 202.6±40.6 for total cholesterol, 50.7±11.0 for HDL-cholesterol, and 130.2±36.7 mg/dL for LDL-cholesterol, respectively. According to the number of risk factors, 7 subjects (3.6%) had 5~6 risk factors, 69 subjects (35.4%) had 3~4 risk factors, 81 (41.5%) subjects had 1~2 risk factors, and 48 subjects (24.6%) had no risk factor. As

Table 1. General characteristics of the subjects

Variable	All (n=195)	Experimental group		
		No risk (n=48)	Low risk (n=115)	High risk (n=32)
Age (yr)	43.9±13.8[1)]	37.5±11.9[b2)]	45.4±13.8[a]	48.1± 13.7[a]
BMI (kg/m^2)	22.9± 3.1	21.7± 2.4[b]	23.2± 3.2[a]	23.8± 3.1[a]
SBP (mmHg)	121.4±13.2	115.1±11.1[b]	122.7±11.6[a]	126.0± 18.1[a]
DBP (mmHg)	73.6± 9.7	69.4± 9.1[b]	73.9± 8.6[b]	78.7± 11.5[a]
Glucose (mg/dL)	93.1±33.0	85.4±13.3[b]	88.8±11.4[b]	120.0± 71.9[a]
Triglyceride (mg/dL)	108.5±75.4	71.8±40.2[c]	106.9±57.5[b]	169.1±122.8[a]
T-cholesterol (mg/dL)	202.6±40.6	187.7±27.9[b]	198.0±38.2[b]	241.1± 40.4[a]
HDL-C (mg/dL)	50.7±11.0	54.2± 8.8[a]	50.4±11.7[ab]	46.7± 9.9[b]
LDL-cholesterol (mg/dL)	130.2±36.7	119.1±24.6[b]	126.3±34.4[b]	160.7± 44.1[a]

[1)]Values are means±standard deviations.

[2)]Values with different superscripts are significantly different at $p<0.05$.

The experimental groups were made by the number of major conventional coronary risk factors the subjects had: no risk, low risk (1~3 risk factors) and high risk (>3 risk factors) groups.

expected, the mean age and BMI were significantly higher in the high risk group than in the no risk group. Also, the blood pressure and fasting glucose levels were significantly higher in the high risk group than in the no risk group. Except HDL-cholesterol that was significantly lower in the high risk group than in the no risk group, plasma triglyceride, total cholesterol and LDL-cholesterole levels were shown to have a similar pattern.

Plasma homocysteine, folate and vitamin B_{12} levels

Plasma homocysteine, folate and vitamin B_{12} concentrations are shown in Table 2. The mean levels of plasma total homocysteine, folate and vitamin B_{12} were 10.36±4.55 μmol/L, 7.20±3.39 ng/mL, and 599.5±275.6 pg/mL, respectively. The plasma homocysteine level was significantly higher in the high risk group than in the no risk group. However, the plasma folate level was significantly lower in the high risk group than in the no risk group. There was no significant difference in plasma vitamin B_{12} levels among the groups.

Frequency distribution of conventional risk factors for cardiovascular disease

The frequency distributions of conventional risk factors for cardiovascular disease are summarized in Table 3. There were 86 (44.1%) subjects with advanced age and 99 (50.8%) male subjects. There were 43 (22.1%) subjects who were obese, 6 (3.1%) subjects with hypertension, 9 (4.6%) subjects with hyperglycemia, and 63 (32.3%) subjects with dislipidemia. The dislipidemia included 37 (19.0%) subjects with hypercholesterolemia, 11 (5.6%) subjects with low HDL-cholesteol, 43 (22.1%) with high LDL-cholesterol, and 8 (4.1%) subjects with hypertriglyceridemia.

Relationship between conventional risk factors for cardiovascular disease and homocysteine, folate and vitamin B12 levels

The relations between conventional risk factors for cardiovascular disease and homocysteine, folate and vitamin B_{12} level are shown in Table 4. The homocysteine level in male subjects was significantly higher than in female ($p<0.001$). The subjects with hyperglycemia ($p<0.05$) had higher plasma homocysteine levels than the subjects with a normal blood glucose level. The subjects with dislipidemia had higher plasma homocysteine levels than the subjects without them ($p<0.05$). The subjects with hypercholesterolemia ($p<0.01$) or high LDL-cholesterol level ($p<0.05$) had higher homocysteine levels than the subjects without those risk factors. However, there was no significant difference in homocysteine levels between subjects with or without hypertriglyceridemia or a low HDL-cholesterol level. In addition, there was no significant difference in plasma homocysteine levels between the subjects with or without other risk factors including advanced age, hypertension, or obesity.

Plasma folate levels in male subjects were significantly lower than in the female subjects ($p<0.01$). However, there were no significant differences in plasma folate levels between subjects with or without other risk factors.

Plasma vitamin B_{12} levels in the subjects with advanced age was significantly higher than in the subjects without advanced age ($p<0.05$). Male subjects had lower plasma vitamin

Table 2. Levels of plasma homocyteine, folate, and vitamin B_{12} according to the number of risk factors

Variables	All (n=195)	Experimental group		
		No risk (n=48)	Low risk (n=115)	High risk (n=32)
Homocysteine (nmol/L)	10.36±4.55[1)]	8.66±1.82[b2)]	10.3±3.8[b]	13.13±7.72[a]
Folate (ng/mL)	7.20±3.39	8.37±3.88[a]	6.86±2.95[b]	6.69±3.77[b]
Vitamin B_{12} (pg/mL)	599.5±275.6	630.7±268.5[ns]	587.3±282.4	597.2±265.8

[1)]Values are means±standard deviations.

[2)]Values with different superscripts are significantly different at $p<0.05$.

The experimental groups were made by the number of major conventional coronary risk factors the subjects had: no risk, low risk (1~3 risk factors) and high risk (>3 risk factors) groups.

Table 3. Frequency distribution of the subjects having risk factors for cardiovascular diseases

Variables	With	Without
Advanced age (male≥45, female≥55 year)	86 (44.1)[1)]	109 (55.9)
Sex (male)	99 (50.8)	96 (49.2)
Obesity (BMI≥25 kg/m^2)	43 (22.1)	152 (77.9)
Hypertension (≥160/95 mmHg)	6 (3.1)	189 (96.9)
Hyperglycemia (≥140 mg/dL)	9 (4.6)	186 (95.4)
Dislipidemia[2)]	63 (32.3)	132 (67.7)
Hypercholesterolemia (≥240 mg/dL)	37 (19.0)	158 (81.0)
Low HDL-cholesterol (<35 mg/dL)	11 (5.6)	184 (94.4)
High LDL-cholesterol (≥160 mg/dL)	43 (22.1)	152 (77.9)
Hypertriglyceridemia (≥250 mg/dL)	8 (4.1)	187 (95.9)

[1)]Values are the number of subjects (%).

[2)]Dislipidemia was defined as the case in which subjects had one or more of the following abnormal lipid levels: hypercholesterolemia (≥240 mg/dL), hypertriglyceriemia (≥250 mg/dL), high LDL-cholesterol (≥160 mg/dL), or low HDL-cholesterol (<35 mg/dL), respectively.

Table 4. Comparisons of the plasma homocysteine, folate, and vitamin B_{12} levels between the subjects with and without each risk factor

		With	Without	P values[2]
Advanced age	Hcy	10.88±4.10[1]	10.2±4.7	Ns
	Folate	7.20±3.50	7.2±3.60	Ns
	Vit. B_{12}	626.0±323.1	588.4±253.5	0.05
Sex (male)	Hcy	11.18±3.88	9.20±2.65	0.001
	Folate	7.43±2.90	8.76±2.82	0.01
	Vit. B_{12}	537.0±222.0	664.0±309.8	0.01
Obesity	Hcy	9.77±2.03	10.18±3.33	Ns
	Folate	7.30±2.77	7.18±3.55	Ns
	Vit. B_{12}	659.3±322.8	582.4±259.2	Ns
Hypertension pressure	Hcy	11.47±2.37	10 05±3.10	Ns
	Folate	7.51±3.98	7.19±3.38	Ns
	Vit. B_{12}	579.1±322.8	600 2±274.9	Ns
Hyperglycemia	Hcy	12.26±4.83	9.98±2.96	0.05
	Folate	8.27±5.74	7.15±3.25	Ns
	Vit. B_{12}	600.4±265.2	599.5±276.8	Ns
Dislipidemia[3]	Hcy	10.86±3.32	9.73±2.92	0.05
	Folate	7.23±3.64	7.19±3.27	Ns
	Vit. B_{12}	636.6±335.7	582.0±241.5	Ns

[1]Values are means±standard deviations.
[2]P values were derived from student t-test.
[3]Dislipidemia was defined as the case in which subjects had one or more of the following abnormal lipid levels: hypercholesterolemia (≥240 mg/dL), hypertriglyceriemia (≥250 mg/dL), high LDL-cholesterol (≥160 mg/dL), or low HDL-cholesterol (<35 mg/dL), respectively.

B_{12} level than female subjects ($p<0.01$). However, there was no significant difference in plasma vitamin B_{12} levels between subjects with and without other risk factors.

The subjects with dislipidemia had higher plasma homocysteine levels than the subjects without them. The subjects with hypercholesterolemia or high LDL-cholesterol had higher homocysteine levels. There were no significant differences in plasma folate and vitamin B_{12} levels between the subjects with and without dislipidemia.

Correlations between homocysteine, folate and vitamin B_{12} levels and cardiovascular risk factors

The correlations between homocysteine, folate and vitamin B_{12} levels and cardiovascular risk factors are shown in Fig 1. The number of risk factors was significantly correlated with plasma homocysteine ($r=0.3137$, $p<0.001$) and the plasma folate ($r=-0.1467$, $p<0.05$). There were no significant correlations in homocysteine, folate and vitamin B_{12} levels with age, BMI, SBP, DBP, total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglyceride levels.

DISCUSSION

Evidence from several epidemiological studies has suggested that elevated levels of homocysteine is an independent risk factor for atherosclerosis. In this study, we investigated the relations of plasma homocysteine, folate and vitamin B_{12} levels with risk factors for cardiovascular disease. First, we compared plasma homocysteine, folate and vitamin B_{12} levels among the groups that were divided according to the number of risk factors, and then compared those levels between the subjects with and without each risk factor for cardiovascular disease.

The plasma homocysteine level was significantly higher in the high risk group than in the no risk group. In contrast, the plasma folate level was significantly lower in the high risk group than in the no risk group. There was no significant difference in plasma vitamin B_{12} levels between high and no risk groups. In other words, the number of risk factors was positively correlated with plasma homocysteine ($r=0.3137$, $p<0.001$), and negatively correlated with plasma folate ($r=-0.1467$, $p<0.05$). Interestingly, the correlation of plasma vitamin B_{12} and the number of risk factors was not significant. It might due to the difference of their importance as cofactors in biochemical homocysteine metabolism. Ueland and Refsum (21) suggested that folate acts as a limiting factor for homocysteine metabolism and folate status is more important than vitamin B_{12} for maintaining normal plasma homocysteine levels. There was no significant correlation between plasma homocysteine, folate, and vitamin B_{12} levels with age, BMI, blood pressure, and lipid levels including total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglyceride levels. Among the conventional risk factors for cardiovascular disease, sex, hyperglycemia and dislipidemia were associated with plasma homocysteine levels. The difference of plasma homocysteine levels between sex is well known in other studies (22-24) and explained by difference in hormonal status (25) or muscle mass (26) between male and female. In this study, sex differences in plasma homocysteine as well as in folate and vitamin B_{12} levels were observed. Males had significantly higher plasma homocysteine levels, while the plasma folate and vitamin B_{12} levels were significantly lower in males than in females. Because plasma folate and vitamin B_{12} levels have been shown to be inversely related to plasma homocysteine levels (13), the facts that male had lower plasma folate and vitamin B_{12} levels also contributes to the difference in homocysteine levels between males and females.

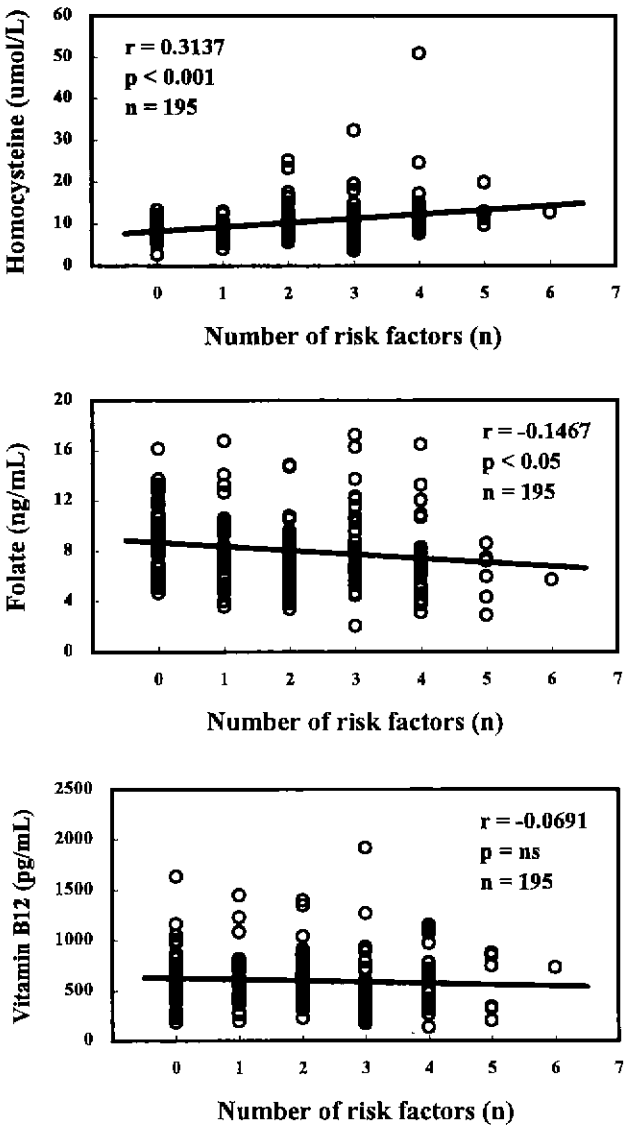

Fig. 1. Relationships of plasma levels of homocysteine, folate, and vitamin B_{12} with the number of risk factors for cardiovascular diseases.

The subjects with dislipidemia had higher plasma homocysteine levels. The subjects with hypercholesterolemia or high LDL-cholesterol had higher plasma homocysteine levels. Despite these results, there was no significant correlation between plasma homocysteine and plasma lipid levels. The present study also showed that there were no significant differences in plasma homocysteine, folate, and vitamin B_{12} levels between the subjects with and without each risk factor including advanced age, hypertension, obesity and hypertriglyceridemia, and there was no significant correlation among them. However, the number of risk factors was positively linked to plasma homocysteine levels and negatively linked to plasma folate levels, which had a negative relation with the plasma homocysteine level. Tsai et al. (17) suggested that because cardiovascular disease is a multifactorial disease, the association between homocysteine levels and each risk factor may be less obvious due to the clustering of various risk factors. They also suggested that homocysteine probably plays a more important role in the early stage of atherosclerosis. Cleophas et al. (16) also suggested that homocysteine may contribute to atherogenesis, which is a very early phase of atherogenesis, and its role might be overshadowed by other risk factors (hypertension, diabetes and lipids).

From these results, we conclude that plasma homocysteine levels might be related to the combination of risk factors, rather than an individual risk factor.

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