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Evaluation of Pasma Folate and Total Homocysteine in Korean Alcoholics

Hyesun Min,† Chang-Su Kim,* Jungsook Seo**

Department of Food and Nutrition, Hannam University, Taejon 306-791, Korea
Teagu Mental Hospital, Taegu 711-830, Korea*
*Department of Food and Nutrition,** Yeungnam University, Kyoungsan 712-749, Korea*

ABSTRACT

Chronic alcoholism often leads to folate deficiency. In recent years it has been reported that mild elevation of plasma homocysteine (Hcy) is associated with an increased risk of coronary artery disease. In the present study we investigated the effects of chronic ethanol consumption on folate status and the relation between plasma folate and Hcy. A human study was conducted to determine plasma folate, total Hcy, cysteine(Cys), total cholesterol and hemoglobin(Hb) concentrations in 44 Korean alcoholics(men aged 30 to 50yr) and 45 Korean non-alcoholic subjects(men aged 30 to 50yr). In alcoholic subjects, 52.6% were folate deficient and 34.2% were marginally deficient, which suggested that most alcoholics were subnormal in folate status. Plasma total Hcy concentration of alcoholics was twice as high as in control subjects($p<0.001$). We found a negative correlation between plasma folate and plasma total Hcy($r=-0.271$, $p<0.05$) and a positive correlation between plasma folate and plasma Cys($r=0.249$, $p<0.05$) in total subjects. Hb concentration in alcoholics was significantly lower than in control subjects, but there was no difference in total cholesterol concentration between alcoholics and controls. These results suggest that chronic alcohol consumption may impair the disposal of Hcy by interfering with folate metabolism. (*J Community Nutrition* 1(1) : 60~65, 1999)

KEY WORDS : folate · plasma total homocysteine · alcoholism.

Introduction

Alcoholism is the most common form of drug abuse in Korea. The chronic ingestion of ethanol is known to affect a variety of organs including gastrointestinal tract, neurological system, hematopoietic system, cardiovascular system and liver. Folate deficiency is prevalent among chronic alcoholics(Eichner & Hillman 1973 ; Herbert et al. 1963 ; Klipstein & Lindenbaum 1965). Previous studies proposed several causes of folate deficiency in severe alcoholism : poor dietary intake(Eichner & Hillman 1971 ; Klipstein & Lindenbaum 1965), intestinal malabsorption(Halsted et al. 1971), increased excretion of urinary folate(McMartin 1984 ; McMartin et al. 1986 ; McMartin et al. 1989 ; Ross et al. 1996), and abnormal hepatic metabolism of folate(Horn et al. 1978 ; Tamura et al. 1981 ; Weir et al. 1985). Chronic ethanol consumption has been reported to interfere with one-carbon metabolism, in which folate serves as a coenzyme(Tamura & Halsted 1983 ; Weir et al. 1985). Ethanol feeding in rats resulted in trapping folate in the liver(Hillman et al. 1977) and the exposure of liver cells to ethanol caused an accumulation of 5-methyltetrahydrofolate in these cells(Horn et al. 1978).

In recent years the association of folate deficiency with mild hyperhomocysteinemia has been confirmed in many studies(Kang et al. 1987 ; Stabbler et al. 1988 ; Ubbink et al. 1993). In addition the evidence of elevated total homocysteine(Hcy) levels as a possible independent risk factor in pathogenesis of coronary artery disease is accumulating. The prevalence of mild hyperhomocysteinemia is higher in the patients with coronary artery disease than in healthy control subjects(Pancharuniti et al. 1994 ; Rubba et al. 1990 ; Ueland & Refsum 1989).

There are three vitamins to serve as precursors of the cofactors for Hcy metabolism. Hcy is catabolized to cystathionine and cysteine by a vitamine B_6-dependent pathway or remethylated to methionine by a folate-and vitamine B_{12}-dependent pathway(Kang et al. 1987 ; Ubbink et al. 1993). Methyltetrahydrofolate is the methyl group

†**Corresponding author** : Hyesun Min, Department of Food & Nutrition, Hannam University, #133 Ojungodong, Taeduk-gu, Taejon 306-791, Korea
Tel : 042) 629-7440, Fax : 042) 629-7490
E-mail : hsmin@eve.hannam.ac.kr

donor in the methionine synthase reaction by which Hcy is remethylated to methionine. Increased methyltetrahydrofolate will enhance the remethylation of Hcy to methionine. Subsequently, this leads to increased levels of s-adenosylhomocysteine(SAM), an activitor of Hcy catabolism (Brattstrom et al. 1988). Therefore, folate may reduce plasma Hcy both by increased remethylation and by increased catabolism.

Low folate concentration in the blood is associated with a mildly elevated total Hcy in alcoholics and non-alcoholics(Cravo et al. 1996 ; Kang et al. 1987 ; Pancharuniti et al. 1994). From the correlation between plasma folate and Hcy concentrations, plasma Hcy concentration has been considered as a sensitive functional indicator of folate status(Kang et al. 1987 ; Stabbler et al. 1988).

Low serum folate levels have been reported in 28 to 80% of recently drinking alcoholics and megaloblastosis has been found even in the presence of normal or borderline-low serum folate levels(Herbert et al. 1963 ; Wu et al. 1975). Since plasma Hcy concentrations are inversely correlated with folate status, low serum folate level in alcoholism is proposed to impair the disposal of Hcy. Cravo et al.(1996) observed that chronic alcoholism is associated with hyperhomocysteinemia and suggested that hyperhomocysteinemia in alcoholics may be related to low RBC folate level. The folate intake and the folate status of Koreans were reported as sub-optimal levels, although the data are very limited(Chang et al. 1999 ; Lim et al. 1999 ; Min & Kim 1996).

In the present study, we examined the relation between folate status and total plasma Hcy concentration and other hematological data in a population of Korean chronic alcoholics and non-alcoholics.

Subjects and Methods

1. Subjects

The subjects were 44 Korean male alcoholic patients hospitalized in mental hospital(aged 30 to 50yr) and 45 Korean non-alcoholic males(aged 30 to 50yr). The alcoholic subjects were currently active drinkers with a history of alcohol consumption for more than 10years. Mean daily ethanol intake of alcoholic subjects was 132.6g/day. Most of these alcoholics drank Korean hard liquor(Soju) more than 3 or 4 times in a week. We attempted to match age distributions in the alcoholic group and the control group. Mean daily ethanol intake in non-alcoholic subjects was 5.2g and they drank no more than the equivalent of 30g of ethanol per day.

2. Collection and preparation of sample

Blood was collected in 0.1% sodium EDTA tube after an overnight fasting(more than 8hr after dinner) and centrifuged for 20min at 2500×g at 4℃ and plasma was separated and stored at −70℃until assay.

3. Biochemical analysis of sample

Plasma folate was assayed by microbiological assay with *Lactobacillus casei* American Type Culture Collection(ATCC 7469, Seattle) as the assay organism. Total plasma Hcy and Cys concentrations were determined by HPLC(Araki & Sako 1987). Total plasma cholesterol and Hb concentrations were analysed with commercial kits(Embiel Co., Korea).

4. Statistical analysis

Student's *t*-test was used for a statistical comparison of means between two groups and the analysis of variance (AVOVA) and Duncan's Multiple Range Test were used to compare means of three groups classified by folate status. Correlation between variables was assessed by Pearson's correlation. Results are presented as means and standard deviations. All significance tests were bilateral and the significance level was set at 5%. All statistical testing was performed using the computer software program SPSS version 8.0 for windows(SPSS, Chicago, IL) for statistics and data analysis.

Results and Discussions

Table 1 showed alcoholic subjects had a significantly lower plasma concentration of folate(8.21±4.08nmol/L) than did the control subjects(11.61±7.63nmol/L) ($p<0.05$). The plasma folate concentration was low in 20 of 38(52.6%) chronic alcoholics but 8 of 45(17.8%) control subjects had low plasma folate(Table 2). Although distribution of plasma folate of alcoholics(2.97−25.43nmol/L) and non-alcoholics(3.67−45.94nmol/L) was overlapped substantially, we observed that plasma folate distribution in non-alcoholics was more widely spread to higher levels(Fig. 1). When we excluded 4 subjects with extremely high plasma folate concentration, significant differences were observed in alcoholics and control subjects(7.88±3.58nmol/L and

Table 1. Baseline data in chronic alcoholics and contol subjects

	Control subjects	Alcoholics
Numbers	45	44
Smoking(%)	62.2	92.9
Age(years)	43.9 ± 7.5	43.0± 7.6
Plasma folate(nmol/L)	11.61± 7.63	8.21± 4.08*
Plasma hcy(μmol/L)	9.54± 2.53	19.03±11.61***
Plasma Cys(μmol/L)	254.3±55.2	237.3±56.8
Hemoglobin(g/dl)	15.0± 1.0	14.4± 1.4
Total cholesterol(mg/dl)	197.4±41.6	185.7±56.6

*p<0.05. ***p<0.001 significant p-value

Table 2. Assessment of plasma folate levels of the subjects

	Criteria of plasma folate[1] (nmol/L)	Control n[2](%[3])	Alcoholics n(%)
Low	<6.8	8(17.8%)	20 (52.6%)
Borderline	6.8 – 11.3	20(44.4%)	13 (34.2%)
Normal	>11.2	17(37.8%)	5 (13.2%)
Total		45(100%)	38[4](100%)

1) Plasma folate levels were assessed by herbert's standard
2) Number of subjects, 3) Percentage of subjects
4) Hemolyzed blood samples were excluded

9.90±3.50nmol/L) (p<0.05). These data demonstrated that folate deficiency is prevalent and a severe problem in alcoholics. Previously Cravo et al.(1996) demonstrated that alcoholism was associated with significant changes of folate concentration in red blood cell but not in serum when compared with control subjects.

Chronic alcoholism is known to be associated with hyperhomocyteinemia, and the reasons for such an increase could be related to a disturbed folate metabolism. In the present study, total plasma Hcy concentrations in alcoholics(19.03±11.61μmol/L) were twice as high as in control subjects(9.54±2.53μmol/L) (p<0.001) (Fig. 2). The mean values of total plasma Hcy concentrations were slightly higher as compared with the data reported previously in other laboratories(Cravo et al. 1996 : Selhub et al. 1993), probably due to storage period difference of blood samples and analytical method of Hcy. Hcy concentration in the blood samples tends to be increased with time at room temperature before separation of plasma by centrifugation. Therefore, the blood samples must be centrifuged immediately after blood sampling(Vester & Rasmussen 1991). The distribution of total plasma Hcy in alcoholics(3.83 – 61.21μmol/L) (Fig. 3) was very widely spread as compared with control group(4.23 – 17.91μmol/L) (Fig. 4). When we employed Hcy concentration above the 95th percentile among 219 normal Korean subjects(unpublished

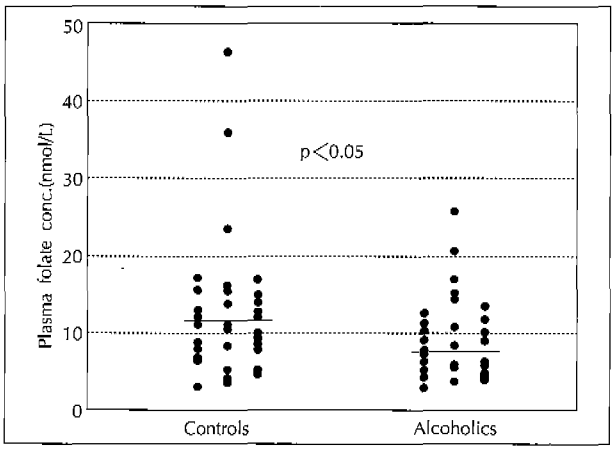

Fig. 1. Distribution of plasma folate concentration in control and alcoholic subjects.
Mean values were significantly different(p<0.05).

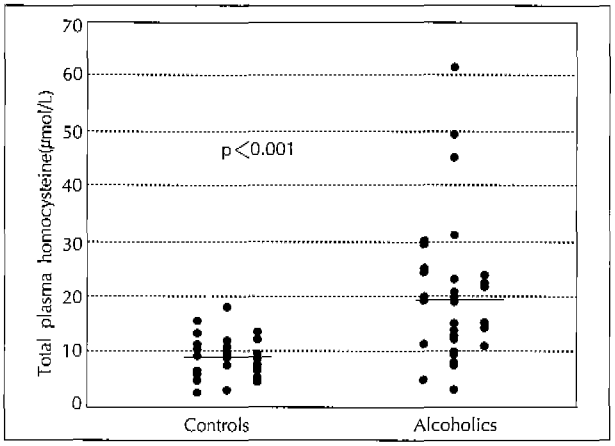

Fig. 2. Distribution of plasma homocysteine concentration in control and alcoholic subjects.
Mean values were significantly different(p<0.001).

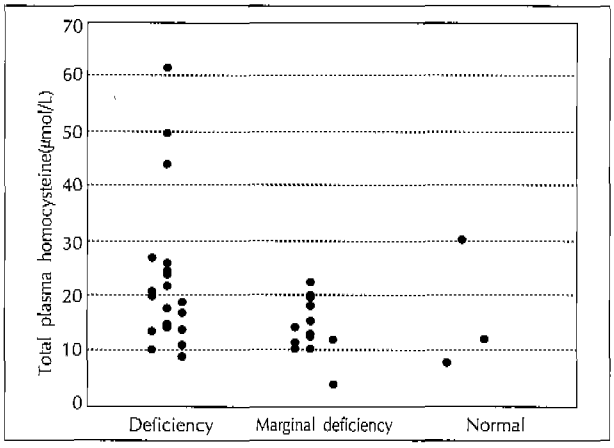

Fig. 3. Distribution of total plasma homocysteine concentration in of total subject in folate deficient, marginally deficient and normal status. Mean values were significantly higher in folate deficient group compared with marginally deficient or normal group.

data) as hyperhomocysteinemia, 14.7μmol/L of plasma Hcy was a cut-off value in this study. Twenty-five of 44 alcoholics(56.8%) were hyperhomocysteinemic and only 2 of 45 control subjects(5%) were mild hyperhomocysteinemics.

Total plasma Hcy, plasma Cys, Hb and total cholesterol of total subjects was compared by folate status(Table 3). Plasma Hcy concentration was significantly higher in the folate deficient group compared with marginally folate deficient or the normal group(Table 3). Therefore, we found that folate deficiency could be a strong cause of hyperhomocysteinemia in this study. Significant differences were not observed in total cholesterol and Hb concentration by folate status although plasma Cys concentrations tended to increase with plasma folate levels($p<0.05$).

Since recent studies reported moderate hyperhomocysteinemia as an independent risk factor of cardiovascular disease, some studies investigated the factors associated with hyperhomocysteinemia(Pancharuniti et al. 1994 ; Shaw et al. 1999). Since plasma Hcy levels were higher in current smokers(Shaw et al. 1999) and most alcoholic subjects in the present study(Table 1) were active smokers(92.9%)(average daily number of cigarettes was 17.4), we could not exclude the effect of smoking on high plasma Hcy levels in alcoholics. For the purpose of excluding the effect of alcohol on plasma Hcy concentration, we compared means of plasma Hcy concentrations of smokers and non-smokers in non-alcoholic subjects. We could not find any significant difference in plasma Hcy concentrations between smokers and non-smokers in this study although the subjects were very limited.

We found a negative correlation between plasma folate and plasma total Hcy($r=-0.270$, $p<0.05$) and the positive correlation between plasma folate and plasma Cys ($r=0.249$, $p<0.05$) in total subjects(Table 4). The inverse correlation between plasma folate and Hcy suggests that a low plasma folate level is responsible for hyperhomocysteinemia. Although hyperhomocysteinemia associated with chronic alcoholism is a direct effect of alcohol or its metabolites interfering with the intracellular metabolism of folate, we could not find a significant correlation between alcohol intake and plasma folate($r=-0.028$, $p=0.879$) or plasma Hcy($r=0.028$, $p=0.879$) in alcoholic subjects. This fact suggests that folate deficiency in chronic alcoholics may be due to not only ethanol ingestion but also nutritional deficiencies. Moreover, vitamin B_6 and vitamin B_{12} deficiencies could be occurring simultaneously in alcoholics, which could contribute separately and independently to the increase of serum Hcy in alcoholics(Cravo et al. 1996). No correlation was found between plasma folate and age or Hb concentration in this study(Table 4).

Recently, several clinical studies have shown that hyperhomocysteinemia in chronic alcoholics could be responsible for an increased incidence of stroke in this group(Gill et al. 1991). The conversion of Hcy to methionine is folate dependent and important for conserving methionine, detoxifying methionine and producing s-adenosylmethionine(SAM) which is the principal methy-

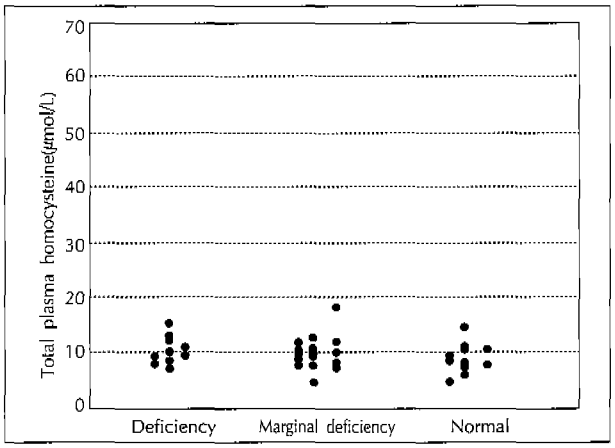

Fig. 4. Distribution of total plasma homocysteine concentration of non-alcoholic subjects in folate deficient, marginally deficient and normal status.

Table 3. Comparison of plasma total hcy, cys, total cholesterol and Hb concentration according to folate status of the subjects

	Criteria of plasma Folate[1] (nmol/L)	Plasma hcy (μmol/L)	Cysteine (μmol/L)	Total Chol. (mg/dl)	Hb (g/dl)
Low	<6.8	18.78±6.22[a2)]	225.4±42.8[b]	175.9±46.0[NS]	14.4±1.5[NS]
Borderline	6.8-11.2	11.17±4.25[b]	249.3±63.5[ab]	190.2±41.6	14.8±1.14
Normal	>11.2	11.07±5.43[b]	262.2±47.9[a]	201.3±54.4	15.1±1.3
Total		13.68±9.21	244.9±54.5	188.6±47.4	14.8±1.3

1) Plasma folate levels were assessed by herbert's standard
2) Values within a column with different superscripts are significantly different at $p<0.05$ by Duncan's multiple range test
NS : not significant

Table 4. Pearson's correlation coefficients between different variables

	Age	Plasma	Plasma	Plasma	Hb
Age	1				
Plasma folate	0.224 (p=0.189)	1			
Plasma Hcy	−0.148 (p=0.403)	−0.271* (p=0.015)	1		
Plasma Cys	0.363* (p=0.023)	0.249* (p=0.025)	0.228 (p=0.163)	1	
Hb	−0.343 (p=0.059)	0.143 (p=0.460)	0.115 (p=0.545)	−0.195 (p=0.295)	1

*p<0.05 significant p-value

lating agent in the organism. Depletion of SAM may promote a membrane injury in alcoholic liver disease(Lieber 1994).

The determinants of elevated levels of Hcy are poorly understood. The variation in plasma Hcy in the population reflects both genetic and nutritional factors. Genetic components seem likely to be involved in some cases(Berg et al. 1992 ; Reed et al. 1991). Particularly, the metabolic defects involved in the metabolism of Hcy can lead to elevations in its levels(Kang et al. 1991 ; Rosenblatt 1995). The heterozygous state for the rare recessive disease of homocystinuria(cystatione-β-synthase deficiency) and autosomal recessive inborn errors of metabolism, such as methylenetetrahydrofolate reductase deficiency, are associated with hyperhomocysteinemia(Kang et al. 1991 ; Rosenblatt 1995). In addition, the existence of genetic traits predisposing to high plasma Hcy levels suggests that some variabilities of plasma Hcy levels in response to folate status might be expected. Since folate, vitamin B_6 and vitamin B_{12} are also involved as cofactors in this metabolic process, low levels of these vitamins can also lead to high levels of Hcy(Brattstrom et al. 1989 ; Kang et al. 1987 ; Ubbink et al. 1993). In the present study, low plasma folate levels in chronic alcoholics is strongly associated with elevated plasma Hcy levels. Since dietary folate intake reflects biochemically measured folate status, an inverse association between Hcy levels and dietary folate would be expected. Nutritional surveys suggest that sub-optimal intake of folate(Kim et al. 1999 ; Lim et al. 1999) and suboptimal blood folate concentration are common in Korean adolescent girls, pregnant and non-pregnant women (Chang et al. 1999 ; Lim et al. 1999 ; Min & Kim 1996).

Elevated Hcy levels can often be normalized by the supplementation of modest doses of folate(1 to 5mg/d) without side effects(Kang et al. 1987 ; Brattstrom et al. 1988). Although various combinations of folate, vitamin B_{12} and vitamin B_6 were often effective in reducing plasma Hcy levels(Dierkes et al. 1999 ; van der Griend et al. 1999), folate appeared to be the effective agent, since folate reduced Hcy levels even when given alone.

In summary, increased plasma Hcy concentration was found in Korean alcoholic men aged 30 to 50yr. Low plasma folate levels appeared to be associated with increased plasma Hcy. Since hyperhomocysteinemia is known to be an independent risk factor of coronary artery disease, excessive alcohol users are recommended to use a larger dose of folate.

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