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Original Article

Study of Methionine, Vitamin B12, and Folic Acid Status in Coronary Atherosclerotic Male Patients

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Abstract

Background: Increased level of serum homocysteine is one of the risk factor of atherosclerosis. Its production related in some sulfur amino acids such as methionine. Some important cofactors that are involved in metabolic pathways of this amino acid are folate and vitamin B12. We have assessed the status of methionine, folic acid, and vitamin B12 in some coronary atherosclerotic male patients.

Methods: In this case-control study, 46 cases of coronary atherosclerosis were selected from male patients aged 37 to 66 years undergoing coronary angiography. Of these, 21 had history of acute myocardial infarction (MI) in previous 3 to 36 months and 25 had angina pectoris. The controls were selected from male healthy volunteers. Inclusion criteria for all study participants required that they had no history of diabetes, hypertension, renal, hepatic, or gastrointestinal disease, endocrinal disorders, or psychiatric illness. Nutritional status was assessed using biochemistry methods and estimation of nutrient intake. Serum methionine was determined by HPLC methods.

Results: Mean serum levels of vitamin B12, and folate, also erythrocyte folate concentration are significantly lower in these patients than in control subjects, but not for methionine. The ratios of serum methionine to vitamin B12 and folate were higher in patients than controls. Vitamin B12 and folate deficiencies, both, were higher in patients than controls.

Conclusion: In summary, it is concluded that, despite normal level of serum methionine, coenzymes deficiencies may be one of the factors accounting for atherosclerosis.

Key words: *Atherosclerosis, Coronary heart disease, Folic acid, Vitamin B12, Methionine*

Introduction

Cardiovascular disease is the main cause of death in most countries (1). It has been shown that risk factors such as obesity, smoking, saturated fat intake and atherogenic plasma lipoprotein profiles contribute to the increase in cardiovascular disease (2-4). However, the role of micronutrients intake as a potential cardiovascular disease risk factor has not been completely examined. Poor dietary habits including deficiencies in several vitamins probably contribute to cardiovascular disease in developing countries (4, 5). Low levels of folate in the diet or plasma are associated with an increased risk of cardiovascular disease (6-9). A mechanism for this association may relate to the inverse association between folate and vitamin B12 status and the concentration of plasma total homocysteine (tHcy), a sulfur containing amino acid formed during the metabolism of methionine (10, 11). Selhub et al. suggested that inadequate plasma concentrations of one or more B vitamins are major determinants of high concentrations of plasma tHcy, a risk factor for cardiovascular disease (12). Folate and vitamin B12 are essential components in the metabolism of tHcy, which occurs through remethylation to methionine or transsulfuration to cysteine. The enzyme methylentetrahydro folate reductase (MTHFR) is responsible for the reduction of 5, 10-methylen-THF to 5-methyl-THF, the required substrate in the remethylation process where vitamin B12 acts as a cofactor (13, 14). The present study attempted to clarify the association between serum level of vitamin B12, folate, methionine and risk of coronary atherosclerosis. Furthermore, we examined the association between folate erythrocyte and risk of atherosclerosis.

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Materials and Methods

Study population Forty six cases of coronary atherosclerosis were selected from male patients aged 37 to 66 yr undergoing coronary angiography. Of these, 21 had history of acute myocardial infarction (MI) in previous 3 to 36 mo and 25 had angina pectoris. The controls were selected from male healthy volunteers. Inclusion criteria for all study participants were no history of diabetes, hypertension, renal, hepatic, or gastrointestinal disease, endocrinal disorders, or psychiatric illness. In the selected study population, 56.5% had no history of vitamins supplements intake, and 13% of the cases and 19.6% of the controls consumed vitamins supplements in two years ago.

The study protocol was approved by the medical ethics committee. All participants gave their written informed consent.

Blood Sampling and Examination Venous blood samples were obtained from all subjects between 7:00 and 8:00 am, after 12 to 14 h fasting. The venous blood obtained was poured onto 3 tubes: First tube containing EDTA for measurement of hemoglobin and hematocrit. To measure the erythrocyte folate, 50µl of this whole blood sample was mixed with 2.5 ml freshly prepared 0.2% ascorbic acid solution (L(+) Ascorbic acid, Cryst, Extra Pure, Merck, FRG), the second tube containing heparin for measurement of triglyceride, total cholesterol and HDL cholesterol after centrifuging and third dry tube for the separation of serum from coagulated blood and measurement of folate vitamin B12 and amino acids. The serum and plasma samples, free of hemolysis, were stored at -70° C before determination of concentrations of triglyceride, total cholesterol, amino acids and vitamin B12.

Dietary Assessment Dietary information was obtained with a 24 h food recall questionnaire during an interview by trained field workers. Dietary analyses were done by use of FP II (Food Processor II, Nutrition Analyses System, ESHA Research) and N-III (Nutritionist III, produced by N-squared computing, oregon, USA) packages.

Biochemical Analysis Plasma triglyceride was measured by using enzymatic colorimetric methods with SERA-PAK kits (Ames SERA-PAK, Triglyceride, Fast color, Miles Ltd. England). Total cholesterol was determined with Leffler modified method (15). To estimate plasma HDL cholesterol, precipitation with magnesium chloride-phospho tangestic acid method was used (16). Radioisotope dilution method was used to determine erythrocyte folate and serum vitamin B12 as well as serum folate with micromedic B12/folate combostt II kits. Serum methionine was separated and determined with Ion Pairs-Reversed Phase High Performance Liquid Chromatography (IP-RP HPLC) with some modifications in Seiler and Knodgen method (17).

Results

Some characteristics of subjects are seen in Table 1. In the age-, sex- matched cases and control subjects, mean serum level of vitamin B12 and folate, and erythrocyte folate, was significantly lower in coronary patients than control group (Table2).

In 73.9% of patients and 97.8% of control subjects, the mean serum level of vitamin B12 was normal (≥150 pg/ml), respectively, although in 26.1% of patients it was low ($P< 0.01$). Mean serum folate levels were low (< 6 ng/ml) in 65.2% of patients and in 50% of control subjects. On the basis of 160 ng/ml cut off point, 6.5% of the cases, but not controls, would have been classified as deficient in erythrocyte folate. Furthermore, mean serum level of both vitamin B12 and folate, in 17.4% of cases and 2.2% of control subjects was low ($P< 0.01$). On the other hand, mean serum level of both vitamin B12 and folate, in 8.7% of cases, but not controls, was marginally low (100-149 pg/ml and 3-5.9 ng/ml respectively). In all the subjects, marginal deficiency of serum folate, more than vitamin B12, was seen (43.5% and 34.8% VS 23.9% and 2.2%). However, a significant difference was observed only for marginal deficiency of vitamin B12 between two groups ($P< 0.01$) (Table 3).

Table 4 shows the mean serum level of methionine, methionine to vitamin B12 ratio, and finally methionine to folate ratio in two groups. Compared with control subjects, cases had statistically significant higher serum ratio of me-

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thionine to vitamin B12 ($P< 0.005$), and non-significant higher serum ratio of methionine to folate, but no significant lower serum methionine. Furthermore, serum methionine correlated positively with serum vitamin B12 (r= 0.45), serum folate (r= 0.40) and erythrocyte folate (r= 0.34).

Relative fluorescence of methionine was lower than valine and tyrosine (Fig.1). As Table 5 shows, vitamin B12 intake was higher in cases, whereas folate intake was lower in cases, no significant. Protein intake correlated positively with vitamin B12 intake (r= 0.78) and, folate intake (r= 0.19).

On the other hand, methionine and cysteine intakes were lower in cases, which are line with protein intake results. Furthermore, protein intake was significantly lower in cases ($P< 0.05$). Compared with Recommended Daily Allowances (RDA), 26.1% of cases and 37% of controls had 75% lower vitamin B12 intake but 73.9% of cases and 63% of controls had 75% lower folate intake. On the other hand, 10.9% of cases and 2.2% of controls had 75% lower methionine intake, compared with WHO recommendations for sulfur amino acids intakes (13 mg/day), but these differences were not significant.

Table 1: Characteristics of cases and controls

Parameters	Cases	Controls
Age(year)	∗51.15±6.70	50.76±6.15
BMI(Kg/m2)	26.46±3.26	26.49±2.99
Total cholestrol/HDL cholestrol	4.94±1.54	4.73±1.55
Smoking status (numbers and percents)	29(63%)	29(63%)

∗ X±SD

Table 2: The mean level of serum vitamin B12 and folate and erythrocyte folate in cases and controls

Parameters	Cases (n=46)	Controls(n=46)	*P*
Vitamin B12(pg/ml)	235.96±129.26∗	353.33±161.51	0.001
Serum folate(ng/ml)	4.97±2.04	6.18±3.21	0.034
Erythrocyte Folate(ng/ml)	284.07±87.46	331.35±112.62	0.027

∗X±SD

Table 3: The relative frequency distribution of serum vitami B12 and folate in cases and controls

Group	Cases(n=46)				Controls(n=46)			
Serum 12(pg/ml)	≤99	100-149	≥150	total	≤99	100-149	≥150	total
Serum folate (ng/ml)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
≤2.9	0(0)	3(6.5)	7(15.2)	10(21.7)	0(0)	1(2.2)	6(13)	7(15.2)
3-5.9	1(2.2)	4(8.7)	15(32.6)	20(43.5)	0(0)	0(0)	16(34.8)	0(0)
≥6	0(0)	4(8.7)	12(26.1)	16(34.8)	0(0)	0(0)	23(50)	23(50)
Total	1(2.2)	11(23.9)	34(73.9)	46(100)	0(0)	1(2.2)	45(97.8)	46(100)

Table 4: The mean level of serum methionine and methionine to vitamin B12 and folate ratios in cases and controls

Parameters	Cases (n=21)	Controls (n=21)	*P*
Serum methionine(μmol/l)	21.22±53.80*	23.85±6.84	NS**
Methionine/vitamin B12 (×10000)	1.71±0.73	1.12±0.44	0.005
Methionine/folate(10)	84.61±42.48	72.42±35.46	NS

*X±SD
**Non Significant

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Table 5: The mean level of energy and some nutrients in cases and controls

Energy and Nutrients	Cases (n=46)	Controls (n=46)	*P*
Energy(Kcal)	2220.09±652.89	2678.61±662.40	0.001
Protein(g)	72.51±25.02	84.27±31.93	0.05
Methionine+Cysteine(mg)	1471.48±645.63	1788.13±1087.71	NS
Vitamin B12(µg)	2.98±2.50	2.71±2.45	NS
Folate(µg)	126.20±83.96	134.22±69.65	NS

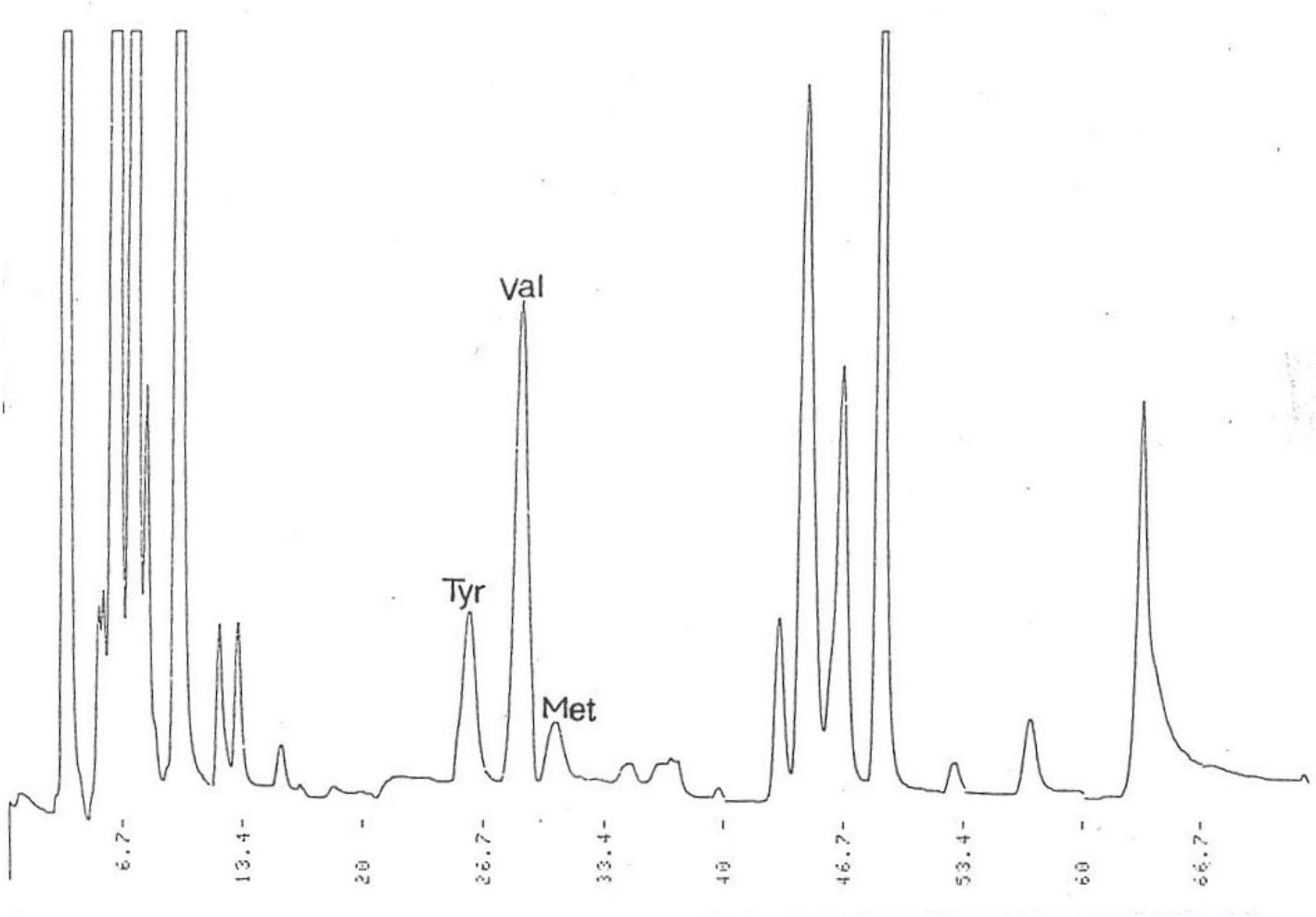

Fig. 1: Relative fluorescence of amino acids

Discussion

Amino acids play a crucial role in metabolism of the vascular endothelium (18). Evidence indicates that dietary sulfur-containing amino acids can exert protective effects on endothelial cell metabolism. Dietary methionine is involved in a variety of potentially protective pathways, such as, protein synthesis, polyamine metabolism, methylation processes or glutation metabolism. On the other hand, methionine is the only dietary source of homocysteine, a potent disrupting agent of endothelial integrity. Thus, an imbalance in dietary methionine may contribute to the development of atherosclerosis (19). Up to date, only

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few reports have indicated that an excess of dietary methionine may contribute to the development of atherosclerosis. Merhova et al. (20) reported that oral methionine administered to rats resulted in an increased number of circulating endothelial cells. Fau et al. (21) observed disturbances in arterial wall morphology in rats chronically fed a diet enriched with 2% methionine. Atherosclerotic changes in methionine-fed rabbits also were reported by McCully and Wilson (22). Conversely, monkeys feeding by a methionine-enriched diet did not induce atherosclerosis (23).

Homocysteine (Hcy) is a sulfur-containing amino acid which originates from demethylation of dietary methionine. The metabolism of Hcy involves remethylation and transsulfuration pathways. The remethylation pathway requires vitamin B12 and folate as coenzymes. Vitamin deficiencies can lead to increased concentrations of tHcy in plasma (24-28). Elevated plasma Hcy levels have been independently associated with an increased risk of atherosclerosis and thrombosis (29-31).Fasting total Hcy concentration has been shown to be a function of age, gender and vitamin status in healthy subjects by several authors (32-34).

On the basis of the negative association between mean serum vitamin B12, folate, and erythrocyte folate levels with total homocysteine in coronary patients, that was confirmed by the majority of studies (35-37), the present study, was conducted to investigate, the methionine and its cofenzymes, vitamin B12 and folate status in coronary patients and healthy control subjects. Our study findings showed that mean level of serum vitamin B12 and folate and erythrocyte folate was significantly lower in coronary patients than control subjects. These findings are consistent with those of Paccharuniti et al. (38), who showed an association between lower folate levels and angiographic evidence of $\geq$ 50% occlusion of one or more major coronary arteries in white males younger than 50 yr of age. Recently, Morrison et al. (37) reported a higher coronary mortality rate in patients with lower folate and vitamin B12 concentrations. In their study, however, no data were available on homocysteine levels. Our findings are consistent with the observation that low functional levels of folate and vitamin B12 that are prevalent in the general population (12), are also common in patients with atherosclerosis. Because it is possible to lower homocysteine levels with folic acid and vitamin B12, such treatments may reduce the risk of atherosclerosis (35). On the other hand, two other prospective studies (37, 39) have shown that low folate status was associated with increased risk of ischemic stroke and coronary heart disease. These studies had no information on tHcy concentrations. However, some factors, e.g., medication, smoking, some diseases, food preparation methods, and intake and absorption, may influence on serum levels of folate and vitamin B12 (40, 41).

In the present study, effects of age and smoking years were omitted, in spite of inverse correlation between mean serum level of vitamin B12 with smoking years (r=-0.21)and matching of two groups in point of age. These findings also showed that folate intake in 74% of cases and 63%of controls, were lower than 75% of RDA. These values for vitamin B12 intake were 26.1% and 37% of cases and controls, respectively. One explanation may be malabsorption. Anyway,"24 h food recall" is not a convenient method, so more exact methods, should be used for vitamin B12 and folate intake, e.g., food records accompanied with food frequency (ffq). In the Robinson et al study, important links between homocysteine, low vitamin concentrations, and vascular disease risk were seen. The causes of hyperhomocysteinemia in those patients were poorly understood, although reduced activity of methionine could play a role. More importantly, however, concentration of homocysteine rise as the levels of folate and vitamin B12 fall, and high homocysteine concentrations were often seen with deficiency of these vitamins (42). In the present study we showed that mean serum level of methionine was not different significantly in two groups and mean serum level of methionine was normal in all the subjects. Our data also revealed that there wasn't difference between two groups in point of sulfur amino aids and methionine. Normal mean serum concen-

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tration of methionine to vitamin B12 and folate ratios in two groups showed that, these ratios were higher in cases than controls, probably due to more efficient remethylation in healthy subjects.

In summary, remethylation of homocysteine, is dependent on folate and vitamin B12 levels, therefore, low folate and vitamin B12 status are important determinants of elevated fasting tHcy levels that is an important risk factor for vascular diseases.

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