Effects of Vitamin B12 and Folic Acid on Hyperhomocysteinemia in Patients with Acute Myocardial Infarction

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The Jordanian population has high rate of coronary artery disease (CAD). The potential association between deficiency of vitamin B12, folic acid, and hyperhomocysteinemia in patients with acute myocardial infarction (AMI), where investigated. A case-control study was carried out involving 210 AMI patients (age 35–70 years; 160 men and 50 women) and 100 normal healthy individuals (age 35–70 years; 70 men and 30 women). Fasting venous blood was obtained from patients and controls. Serum was analyzed for vitamin B12 and folic acid using radioassays. The mean serum B12 concentration in AMI patients was found to be significantly lower in controls. The mean serum folate level in patients was also found to be lower than in controls. On the contrary, plasma homocysteine level in AMI patients was higher than that in controls, but not significantly. However, the homocysteine in normal healthy controls was greater than what had been reported in the literature. Vitamin B12 and folate deficiency in AMI patients was significantly higher than in controls. Plasma levels of homocysteine in smokers were also significantly higher in both patients and controls. Additionally, smokers had significantly lower serum folate levels than nonsmokers. Vitamin B12 and folate deficiency in association with hyperhomocysteinemia may be considered as a risk factor for CAD development.

Key words —— cardiovascular disease, acute myocardial infarction, hyperhomocysteinemia, vitamin B12, folate

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death in both developed and developing countries. A number of studies during the past few years have indicated a protective role of vitamin B12 and folate against the development of CAD. [1] More recent reports have shown an association between the deficiency of vitamin B12 and hyperhomocysteinemia, a known risk factor for myocardial infarction.[2] This has focused attention on vitamin B12 and folate and the important role they might play in protection against the development of CAD.[3]

An elevated plasma homocysteine level is due to congenital or acquired defects in the pathway of homocysteine metabolism. The conversion of homocysteine to methionine is vitamin B12 dependent in which folate is required as a methyl group donor.[4] Thus the plasma level of homocysteine is dependent on activities of key enzymes of the metabolic pathway and the intake of vitamin B12 and folate.[5, 6]

Homocysteine is present in plasma as protein-bound and free forms. The majority of homocysteine is bound to albumin, while the free form exists as homocysteine disulfide, homocysteine-cysteine mixed disulfide, and trace amounts of reduced homocysteine.[7] The reduced homocysteine form increases in patients with severe homocysteinemia. In renal tubular cells and hepatocytes, homocysteine levels are regulated by two transmethylation and a transsulfuration pathways. Human aortic endothelial cells lack those pathways.[8] Therefore, human vascular endothelium has a limited capacity to metabolize homocysteine, leaving it more susceptible to homocysteine-induced injury than other tissues. In addition, hyperhomocysteinemia is associated with the risk of severe coronary atherosclerosis and therefore considered an independent risk factor for coronary vascular disease similar to hypertension, hyperlipidemia and smoking.[9]

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The objectives of this research were to evaluate the relationship between plasma homocysteine levels and ischemic heart disease and to investigate whether patients with acute myocardial infarction (AMI) have lower levels of plasma B12 or folate and increased homocysteine levels compared with healthy individuals.

MATERIALS, PATIENTS AND METHODS

Two hundred and ten patients, aged 35–70 years, with a confirmed diagnosis of AMI and normal renal function (serum creatinine < 100 μmol/l) were included in this study. Subjects were selected from patients admitted to the coronary care unit of Al-Amir Rashid Hospital, Irbid, Jordan, during the period from March to August 2002. The selection was based on the AMI WHO (World Health Organization) criteria: clinical history of myocardial ischemia, ECG findings, and elevation of biochemical markers. Patients were also assessed for risk factors for CVD, such as hypertension, hypercholesterolemia, diabetes mellitus, obesity, smoking, and a family history of ischemic heart disease (IHD). Exclusion criteria for patients as well as for controls were: pregnancy, use of antiepileptics, oral contraceptives, malabsorption syndrome, tuberculosis, liver disease, uremia, cancer, and the use of vitamin B-complex supplements during the past 6 months. All blood samples were obtained at least 3 months following AMI.

Similarly, 100 aged and sex-matched healthy individuals were selected as controls and screened for the above risk factors. Exclusion criteria for controls also included evidence of CAD, diabetes mellitus, hypertension, obesity, and hypercholesterolemia.

Informed consent was obtained from all participants and the study was approved by the Ethics Committee of Jordan University of Science and Technology School of Medicine.

Fasting serum glucose, total cholesterol, High Density Lipoprotein cholesterol, and triglycerides were analyzed from venous blood obtained within 24 hr of AMI, using a commercial colorimetric kit (Randox, Antrim U.K.). The Friedewald formula was used to calculate the concentration of Low density Lipoprotein cholesterol.[10] Serum samples were analyzed for vitamin B12 and folate using radioassays.[11, 12] Plasma homocysteine was determined using a commercial kit based on the fluorescence polarization immunoassay (Abbott Laboratories, Ltd., Hamburg Germany).

Statistical analysis values are reported as mean ± S.D. Percentages were compared with the test of proportions using chi-square. Mean values of various groups were compared using Analysis of Variance. The analyses were performed using SPSS software version 9 (SPSS Inc., Chicago, IL, U.S.A.). A P value of less than 0.05 was considered significant.

RESULTS

Table 1 describes the characteristics of both the control group and AMI patients. Controls were 70 men and 30 women, with a mean age of 53.28 ± 6.82 (range 35–70) years. The mean BMI was 26.78 ± 2.68 kg/m^2 and 10 controls (10%) were obese. Forty-two percent of the controls were smokers. Eighteen percent of the controls had a family history of IHD. Mean serum levels of glucose, total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides were 102 ± 34 mg/dl, 165 ± 65 mg/dl, 36.4 ± 7.5 mg/dl, 97 ± 46 mg/dl and 172 ± 95 mg/dl, respectively. AMI patients were 160 men and 50 women. The mean age was 51.81 ± 7.54 years (range 35–70) years. Mean BMI was 27.56 ± 3.45 kg/m^2. Twenty-five patients (11.9%) were obese, 49% were smokers, 60% were hypertensive, 48% were diabetics and 30% had hypercholesterolemia. Twenty nine percent of AMI patients had a family history of IHD. Mean serum levels of glucose, total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides were 148 ± 78 mg/dl, 190 ± 68 mg/dl, 31.5 ± 12.4 mg/dl, 119 ± 78 mg/dl, and 185 ± 120 mg/dl, respectively.

Mean levels of serum B12, serum folate, and plasma homocysteine in the healthy controls and AMI patients are shown in Table 2. The mean serum B12 level in AMI patients (264 ± 154 pg/ml) was significantly ($p < 0.005$) lower than that in healthy controls (615 ± 285 pg/ml). The mean level of vitamin B12 was also significantly ($p < 0.025$) higher in women than in men in both control and patient groups. Even though the mean serum folate level in AMI patients (3.14 ± 3.56 ng/ml) was lower than that in controls (4.85 ± 1.95 ng/ml), this difference was not significant. Also mean levels of folate in

Table 1. Characteristics of Healthy Individuals and AMI Patients

	Controls (n = 100) (mean ± S.D.)	Frequency (%)	Patients (n = 210) (mean ± S.D.)	Frequency (%)
Age (years)	53.28 ± 6.82		51.81 ± 7.54	
Sex				
Male		70 (70)		160 (76)
Female		30 (30)		50 (24)
BMI (kg/m^2)	26.78 ± 2.68		27.56 ± 3.45	
Smoking		42 (42)		103 (49)
FH of IHD		18 (18)		61 (29)
Hyperchol.		0		63 (30)
HTN		0		126 (60)
DM		0		101 (48)
Glucose (mg/dl)	102 ± 34*		148 ± 78*	
Total-chol (mg/dl)	165 ± 65*		190 ± 68*	
HDL-chol (mg/dl)	36.4 ± 7.5		31.5 ± 12.4	
LDL-chol (mg/dl)	97 ± 46*		119 ± 78*	
Triglycerides (mg/dl)	172 ± 95*		185 ±120*	

BMI: Body mass index; FH of IHD: Family history of ischemic heart disease; Hyperchol.: hypercholesterolemia; HTN: hypertension; DM: diabetes mellitus. * $p < 0.05$.

Table 2. Serum or Plasma Levels of Vitamin B12, Folate and Homocysteine in Controls and AMI Patients

Variables	Controls			AMI patients		
	All	Men (n = 70)	Women (n = 30)	All	Men (n = 160)	Women (n = 50)
Vitamin B12 (pg/ml)	615 ± 285*	598 ± 298*	671 ± 324*	264 ± 154*	218 ± 146*	284 ± 185*
Folate (ng/ml)	4.85 ± 1.95*	4.65 ± 2.32*	5.82 ± 2.78*	3.14 ± 3.56*	2.45 ± 2.1*	5.12 ± 4.88*
Homocysteine (μmol/l)	17.38 ± 3.78	16.92 ± 4.2	15.1 ± 3.8	19.28 ± 7.4	19.65 ± 7.2	14.68 ± 4.8

Data reported as mean ± S.D. Controls *vs.* AMI patients, * $p < 0.005$ (all) and * $p < 0.03$ (sex).

Table 3. Deficiency of Vitamin B12 and Folate in Controls and AMI Patients

Group	B12 deficiency % (n)	$p^{a)}$	Folate deficiency % (n)	$p^{a)}$
Controls (n = 100)	5 (5)		29 (29)	
		0.05		0.0005
AMI patients (n = 210)	69.5 (146)		71 (149)	

[a)] p value compares percentage in controls and AMI patients.

all men were lower than that in all women, but this difference was not statistically significant. The mean homocysteine level in AMI patients (19.28 ± 7.4 μmol/l) was higher than that in controls (17.38 ± 3.78 μmol/l) and all men had mean homocysteine levels higher than that in women. However, these differences were not statistically significant.

While a smaller percentage of healthy controls had vitamin B12 and folate deficiencies (5% and 29%, respectively), AMI patients were significantly deficient in both vitamin B12 and folate (69.5% and 70.95%, respectively). This may suggest a positive association between deficiencies of those vitamins and IHD (Table 3).

The relationship between plasma homocysteine levels and vitamin B12 and folate is described in Table 4. Mean homocysteine levels in vitamin B12 deficient and folate-deficient groups of AMI pa-

Table 4. Mean Values of Plasma Homocysteine Relative to Vitamin B12 and Folate Sstatus in Controls and AMI Patients

	Controls (n = 100)		$p^{a)}$	AMI patients (n = 210)		$p^{a)}$
	No. (n)	Homocysteine (μmol/l)		No. (n)	Homocysteine (μmol/l)	
B12						
Normal	97	17.34 ± 4.24	0.5	75	14.2 ± 4.5	0.0005
Deficiency	3	21.28 ± 8.84		135	20.24 ± 7.8	
Folate						
Normal	68	16.42 ± 2.8	0.0005	68	14.46 ± 4.8	0.0005
Deficiency	32	20.14 ± 4.8		142	20.34 ± 6.8	

a) p value compares means of homocysteine in vitamin-normal and -deficient groups for both controls and AMI patients.

Table 5. Serum or Plasma Levels of Vitamin B12, Folate and Homocysteine in Smokers and Nonsmokers in Control and AMI Patient Groups

	Smokers	Nonsmokers	$p^{a)}$
Controls			
B12 (pg/ml)	595 ± 210	618 ± 240	0.25
Folate (ng/ml)	3.8 ± 3.15	6.34 ± 3.24	0.02
Homocysteine (μmol/l)	20.4 ± 6.14	16.25 ± 5.2	0.042
AMI patients			
B12 (pg/ml)	210 ± 145	285 ± 205	0.007
Folate (ng/ml)	2.78 ± 3.19	4.25 ± 4.76	0.0045
Homocysteine (μmol/l)	20.64 ± 7.8	17.28 ± 8.2	0.03

a) p value compares means in smokers and nonsmokers.

tients were significantly increased (p = 0.0005, p = 0.0005, respectively) in comparison to the mean levels in vitamin B12-normal and folate-normal patient groups. Mean homocysteine levels in B12-deficient individuals in the control group were also higher than those in the B12 normal individuals in the same group. Nonetheless, those levels did not reach statistical significance (p = 0.5). Additionally, mean homocysteine levels in the folate-deficient group in the controls were significantly (p = 0.0005) higher than those in the folate-normal control group.

No significant difference in mean levels of B12, folate, and homocysteine between older and younger groups was seen when controls and patients were divided into two age-groups: older and younger than the mean age of both patients and controls 52.6 years.

Table 5 compares the mean level of vitamin B12, folate, and homocysteine in smokers and nonsmokers in both controls and AMI patients. Smokers had lower levels of vitamin B12 and folate and higher levels of homocysteine than nonsmokers in both the control and AMI groups. All these differences were statistically significant, except for vitamin B12 levels in the control group. High homocysteine levels in smokers might be due to the low levels of folate.

DISCUSSION

Mortality due to CAD is a global problem.[13)] Despite the lack of accurate data on the mortality rate from CAD in Jordan, AMI was reported to be the leading cause of death.[14)] Furthermore, the prevalence of diabetes mellitus, impaired glucose tolerance, and hypertension in Jordan has been reported to be 13.4%, 9.8%,[15)] and 16.3%, respectively.[16)] Therefore, and due to the high prevalence of CAD risk factors we investigated the effect of vitamin B12 and folate deficiencies and plasma levels of homocysteine in AMI patients.

Table 3 shows a significant association between vitamin B12 and folate deficiencies and the development of CAD. This may suggest that deficiency in those vitamins may play a critical role in the development of CAD. On the other hand, homocysteine levels in AMI patients were not significantly

different form those in controls (Table 2). Vitamin B12 deficiency in our AMI patients (69.5%) is considered extremely high. Because our population is mostly nonvegetarian, it is thought that B12 deficiency would be less prevalent than folate deficiency. However, our data indicate that B12 deficiency is very common in our AMI patients. Because pernicious anemia is not very common in Jordan, it is possible that the main factor responsible for B12 deficiency is inadequate absorption.

The high prevalence of folate deficiency in the control group (29%) was relatively unpredicted (Table 3). This might imply that folate deficiency is common. High prevalence of folate deficiency in both controls and AMI patients could be due to insufficient consumption of fresh fruit and vegetables. Even though hyperhomocysteinemia was seen in AMI patients (Table 2), it cannot be considered independent risk factor for development of CAD. Our data indicate that B12 and folate deficiencies may have an association with hyperhomocysteinemia (Table 4), even though it could arise from numerous factors, considering that both controls and AMI patients were from a relatively low socioeconomic class and possibly had inadequate nutrition. Interestingly, homocysteine levels in controls were higher than the normal homocysteine levels (5–15 μmol/l) reported in several studies.[17,18] Such elevated homocysteine levels in healthy individuals were reported in India,[19] Thailand,[20] and Turkey.[21] These findings might explain our previously reported high rate of CAD in Jordan especially among younger males.[14] Nevertheless, the increase in homocysteine levels in our AMI patients was not significant. The negative correlation between the development of CAD and homocysteine levels in our study should not be considered odd, since the degree of correlation between CAD and homocysteine levels may differ from population to population depending on genetic factors and gene-environment interaction. It has been reported that the genetic background of the Finnish differs from that of other countries.[22] Consequently, the negative correlation between CAD and homocysteine levels in the Finnish is reasonable, even though, it is not in agreement with several reports from other countries.[22] Furthermore, recent reports have indicated that folic acid and vitamin B6 and B12 treatment did not lower the risk of cardiovascular events in patients with cardiovascular disease.[23–25]

Increased plasma homocysteine levels in the elderly have been reported.[26–29] Nevertheless, our data did not demonstrate a marked difference from mean values of plasma homocysteine in individuals younger than 53 years even though an increase in homocysteine levels was observed in those older than 53 years.

Our findings of significantly lower levels of folate in smokers in comparison with nonsmokers in both AMI patients ($p = 0.0045$) and controls ($p = 0.02$), are in agreement with those reported by Mansoor *et al.*[30] who demonstrated a significant decrease in the levels of folate in smokers. Free radicals from cigarette smoking, inadequate consumption of vegetables and fruit, and increased excretion of folate might have participated in decreased body folate.[31–33] In our research, homocysteine levels in smokers were significantly higher than the levels in nonsmokers, which correlated with low levels of vitamin B12 and folate. Furthermore, increased homocysteine levels in smokers compared to nonsmokers was reported in a large community study in Norway.[32]

Based on our findings, we may propose that vitamin B12 and folate deficiencies as well as increased levels of plasma homocysteine could aggravate the risk of CAD development.

There was a deficiency in vitamin B12 and folate in our study group. The deficiency in those vitamins accompanied by increased levels of plasma homocysteine was even more prominent in the AMI patient group. This may explain the high rate of CAD among Jordanians and might imply the protective role of those vitamins against the development of CAD. However, our study was a small-scale, hospital-based and a larger-scale, community-based study will probably further explore the role of hyperhomocysteinemia and vitamin B12 and folate deficiencies in the development of CAD.

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