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ORIGINAL ARTICLE

Role of Homocysteine, Vitamins B6, B12 and Folic Acid in Acute Myocardial Infarction Patients

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ABSTRACT

Background: Homocysteine is a risk factor for cardiovascular disease. We evaluated the efficacy of homocysteine-lowering treatment with B vitamins for secondary prevention in patients who had had an acute myocardial infarction.

Methods: We investigated the possible correlation between deficiency of vitamins B6, B12 or folic acid and homocysteine in patients with acute myocardial infarction (AMI). A case control study was carried out involving 50 AMI patients and age matched 50 normal healthy subjects.

Results: Mean serum B12 concentration in AMI patients was found to be significantly lower than the mean for controls. Mean serum folate and PLP level in patients was also found to be lower than controls; however, the differences were not statistically significant. Mean plasma homocysteine level in AMI cases was higher than the mean level in controls. Compared to controls, there was significantly greater deficiency of folate, B12 and PLP in AMI patients.

Conclusion: Substantial nutritional deficiencies of these three vitamins along with mild hyperhomocysteinemia, perhaps through interplay with the classical cardiovascular risk factors (highly prevalent in this population), could be further aggravating the risk of CAD in the population.

Key Words: Folic acid, vitamin B6, vitamin B12, homocysteine, AMI

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Introduction

Cardiovascular disease (CVD) is the leading cause of death in both industrialized and developing countries. Recent reports have indicated that Indian people belong to an ethnic group, which has the highest rates of coronary artery disease (CAD) [1]. Moreover, the relative risk of CAD in South Asian men is highest at early ages and suggesting that both intrinsic and extrinsic factors contribute to the development of CAD in this population [2], [3].

A number of studies during the past few years have indicated a protective role of vitamins B6, B12 and folate against the development of CAD [4], [5], [6]. More recent reports have shown an association between deficiency of these B-complex vitamins and hyperhomocysteinemia, a known risk factor for myocardial infarction [7], [8]. This has focused attention on these

B-complex vitamins and the important role they might play in protection against the development of CAD [9].

The objective of this study is to investigate whether or not our patients suffering from acute myocardial infarction (AMI) have lower levels of plasma B6, B12 or folate and homocysteine compared to healthy subjects. If deficiency of B-complex vitamins is found to exist in this population, then intervention in the form of supplementations might significantly reduce the incidence of CAD.

Materials and Methods

Study Population

The populations of study consider two hundred subjects, divided in two groups. Hundred AMI patients (age 30–70 years) admitted to the Kovai Medical Centre and Hospital (KMCH) & K.G. Hospital and Post Graduate Medical Institute Tamil Nadu from January 2007 to February 2008 were included in this study. They were enrolled within the index admission after confirmation of diagnosis based on WHO criteria of clinical history suggestive of myocardial ischemia, ECG indications of myocardial damage, and elevation of biochemical markers (creatine kinase and creatine kinase-MB). All patients were assessed as having risk factors for CVD, such as diabetes mellitus, hypertension, obesity, hypercholesterolemia, smoking and a parental history of ischemic heart disease (IHD). Criteria for diabetes were set as an abnormal fasting blood glucose level >125 mg/dl at admission, or having taken hypoglycemic agents. All those with systolic blood pressure greater than 140 mmHg and/or diastolic blood pressure of 90 mmHg or those on regular anti-hypertensive medications were classified as hypertensive. Those having serum cholesterol level greater than 200 mg/dl were considered to have hypercholesterolemia. A body mass index (BMI) above 30 was classified as obese, and a parental history of IHD was considered positive if any of the parents had IHD at or below the age of 60 years. Subjects were considered smokers if they had been smoking cigarettes regularly (one or more per day). Those cases (as well as controls) that were pregnant, using anti-epileptics, taking oral contraceptives, having malabsorption syndrome, suffering from tuberculosis, liver disease, uremia, or cancer or using vitamin B-complex supplements during the past 6 months were not included.
Similarly, age and sex matched 100 normal healthy subjects were also investigated. They were also assessed for the above-mentioned risk factors. However, more stringent criteria were used for the selection of normal healthy control subjects. In addition to being matched for age, sex and socioeconomic background, they had no evidence of CAD, diabetes mellitus, hypertension, obesity or hypercholesterolemia. The objective was to have an assessment of the levels of folate, B12, B6 and homocysteine in a "normal healthy population" and compare their levels with those obtained from AMI patients.

Estimation of Biochemical Investigation

Biochemical investigation including blood glucose fasting, total cholesterol, triglycerides, HDL, LDL and CK levels were determined by fully automated clinical chemistry analyzer (Hitachi 912, Boehringer Mannheim, Germany). VLDL level was calculated according to Friedewald *et al*. [10] CK-MB mass were measured with highly specific monoclonal antibodies in a sensitive chemiluminescence assay, with an Elecsys 2010 instrument (Roche Diagnostics, Mannheim, Germany). Troponin I level was estimated using fully automated immunoassay analyzer (AXSYM-Abbott Laboratories, Abbott Park, USA).

Estimation of B12, folate, B6 and homocysteine in serum/plasma

Fasting venous blood was obtained from cases as well as controls. Serum samples were analyzed for folate and vitamin B12 using radio assays [11,12]. Plasma samples from both cases and controls were screened for pyridoxal phosphate (PLP; coenzymic form of vitamin B6) and homocysteine. Plasma PLP concentration was chosen as the standard for vitamin B6 status because this measure appears to reflect tissue stores Lui [13]. For determination of PLP in plasma, a modification of the method by Camp et al. [14] as described previously was used Iqbal et al [15]. Determination of plasma homocysteine was carried out using a kit based on fluorescence polarization immunoassay (Abbott Laboratories, Ltd., USA).

Statistical analysis

All data were expressed as mean ± SD. The statistical significance was evaluated by Student's *t* test using Statistical Package for the Social Sciences (SPSS Cary, NC, USA) version 10.0.

Results

Demographic data of control and AMI group are shown in [Table/Fig 1]. The mean age limit was 47.5 ± 12.5 years in AMI patients and 43.7 ± 10.3 years in control subjects. The increased body mass index (BMI) in AMI patients (28.8 ± 4.2 kg/ m^2) when compared to control subjects (23.2 ± 3.7 kg/ m^2) was statistically significant. Blood Pressure systolic blood pressure was significantly high ($p<0.05$) in patients groups as compared with controls.

(Table/Fig 1) Demographic and clinical characteristics of subjects

Parameter	Control subjects	AMI patients
Age (Mean ±S.D) years	43.7 ± 10.3	47.5 ± 12.5
Sex (Males %)	50[100%]	50[100%]
Body mass index	23.2 ± 3.7	28.8 ± 4.2**
Risk factors, %		
Hypertension	-	32[76]
Cardiovascular disease	-	18[24]
Smoking status		
Current smoker	16[31%]	32[63%]
Ex-smoker	10[20%]	12[25%]
Non-smoker	24[49%]	6[12%]
Dietary habits		
Olive oil	13.5[29.5%]	19[37.5%]
Olive oil + margarine	36.5[70.5%]	31[62.5%]

Values are given as mean ± S.D from 100 subjects in each group.

AMI patients compared with control subjects. (**p<0.001, *p<0.05)

The comparison of biochemical changes in control and MI subjects are shown in [Table/Fig 2]. As expected, the patients had significantly higher level of total cholesterol, triglyceride, LDL-cholesterol and VLDL-cholesterol when compare to control subjects, but HDL-cholesterol in AMI patients was significantly less than in normal healthy subjects. There was no statistical significant in fasting blood glucose. The significantly increases in the level of CK, CK-MB seen in MI patients when compare to control subjects.

(Table/Fig 2). Levels of serum lipids and cardiac markers in control and acute myocardial infarction subjects

Parameter	Control subjects	AMI patients
Fasting Glucose (mg /dl)	97.81 ± 7.03	100.24 ± 10.1^{NS}
Total cholesterol (mg/ dl)	154.20 ± 10.7	231.23 ± 21.8***
Triglyceride (mg/dl)	100.37 ± 12.18	175.67 ± 10.25***
HDL-cholesterol (mg /dl)	48.85 ± 5.37	37.80 ± 7.15*
LDL-cholesterol (mg /dl)	76.65 ± 9.84	137.02 ± 10.90***
VLDL-cholesterol (mg /dl)	27.70 ± 3.00	39.72 ± 7.40***
CK (IU /L)	73 ± 15.6	195 ± 12.1***
CK-MB (IU/ L)	12.5 ± 2.8	29.5 ± 4.3***

Values are given as mean ± S.D from 50 subjects in each group.
AMI patients compared with control subjects.
(*p<0.05, ***p<0.001,NS-Not significant)

[Table/Fig 3] shows the mean concentrations of serum folate, serum B12, plasma PLP and plasma homocysteine in normal healthy subjects (controls) and AMI patients. Analysis the mean serum B12 concentration in AMI patients was significantly less than in normal healthy subjects. Mean serum folate level in AMI patients was found to be lower than the mean level in normal healthy control subjects. Mean plasma PLP concentration in AMI patients was also found to be lower than the controls, however, the values were not significantly different. Mean homocysteine concentration in plasma of AMI patients was higher compared to controls (11.2±4.02 μmol/l vs 19.5 ±7.85 μmol/l).

(Table/Fig 3). Concentration values of serum/plasma folate, B12 and homocysteine in normal healthy subjects and AMI patients (mean values ± SD)

Parameter	Control subjects	AMI patients
Folate (ng/ml)	5.33 ± 2.70	3.29 ±1.75**
Vitamin B12 (pg/ml)	656 ±327	225 ±137***
PLP (nmol/l)	22.4 ±15.8	20.5 ±13.6 NS
Homocysteine (μmol/l)	11.2 ±4.02	19.5 ±7.85***

Values are given as mean ± S.D from 50 subjects in each group. AMI patients compared with control subjects. (**p<0.01, ***p<0.001)

Discussion

Significantly high percentages of folate, B6, and B12 deficiencies in AMI patients in our study population suggest a causal relationship between nutritional deficiency of these vitamins and development of CAD. The high prevalence of folate and B6 deficiencies in our control group was quite unexpected. With hardly any data available on deficiency of these two vitamins in the general population, our results indicate that folate and B6 deficiencies could be quite common. High prevalence of folate deficiency in our normal as well as patient populations could be due to our urban dietary habits, which include inadequate use of fresh fruit and vegetables and overcooking. There has been a considerable debate on the value of plasma PLP which should be taken as standard for adequate B6 status [16].

Although a mild hyperhomocysteinemia has been observed in our patients suffering from AMI, it does not appear to be an independent risk factor for development of CAD. The causes of hyperhomocysteinemia are multifactorial, but our preliminary data indicate that folate deficiency and B6 deficiency certainly appear to have an association with it. This could be due to the fact that most of the subjects in our cases and control populations belonged to the lower middle class, a group with a relatively modest socio-economic background and perhaps receiving somewhat poor and unbalanced nutrition [3].

A number of studies have shown increased plasma homocysteine levels in aged individuals [17], [18]. Our observation of significantly lower levels of serum folate in smokers compared to nonsmokers in the normal control group, as well as in the AMI patients group, is consistent with the results reported by Mansoor et al. [19] who showed a significant decrease in both serum and erythrocyte folate in smokers. Lower intake of fruit and vegetables, free radicals in cigarette smoke, and increased excretion of folate might have contributed to a decline in body stores of folate [20]. With low folate and low B12 concentrations in smokers, mean plasma homocysteine concentrations among smokers (both in controls as well as AMI patients), as expected, were found to be significantly higher than in nonsmokers. Nygard et al. [21] in a study involving nearly 16,000 subjects from a general community in Norway have also reported significantly increased levels of total homocysteine in smokers compared to nonsmokers.

On the basis of these observations, it can be suggested that very significant deficiencies of folate, B6 and B12 along with mild hyperhomocysteinemia could act synergistically in concert with other classical risk factors in the study-population, thereby further aggravating the risk of CAD.

Our study reports the prevalence of folate, B6 and B12 deficiency and its relationship

with plasma homocysteine levels. Our data show substantial nutritional deficiency of all these three vitamins. Deficiencies of folate, B6 and B12 were even more pronounced in AMI patients, consistent with their important role in preventing the development of CAD. Any single nutritional intervention, such as supplementation with folate alone, may not be enough to protect against CAD in a population that has the highest rates of this disease compared to any other in the world. However, supplementations with all three vitamins would, perhaps, offer some protection. The association between B-complex vitamin deficiency, hyperhomocysteinemia, other classical risk factors and the risk of developing CAD merits our serious attention.

References

[1] Ranucci M, Ballotta A, Frigiola A, Boncilli A, Brozzi S, Costa E, Mehta R H. Pre-operative homocysteine levels and morbidity and mortality following cardiac surgery. Eur Heart J 2009; 30: 995-1004.

[2] McKeigue PM. Coronary heart disease in Indians, Pakistanis and Bangladeshis: etiology and possibilities of prevention. Br Heart J1992; 67: 341-42.

[3] Iqbal MP, Ishaq M, Kazmic KA, Yousuf FA, Mehboobali N, Ali SA, Khan AH, Waqar MA. Role of vitamins B6, B12 and folic acid on hyperhomocysteinemia in a Pakistani population of patients with acute myocardial infarction. Nutrition Metabolism and Cardiovascular Diseases 2005; 15: 100e-108e.

[4] Losonczy KG, Harris TB, Havlik RJ. Vitamin E and vitamin C supplement use and risk of all-cause and coronary heart disease mortality in older persons: the established populations for epidemiological studies of the elderly. Am J Clin Nutr 1996; 64:190-96.

[5] Ebbing M, Ueland PM, Nygard O. Lowering homocysteine with B vitamins in patients with coronary artery disease-Reply. JAMA 2008; 300: 2853-2854.

[6] Hodis HN, Mack WJ, Dustin L, Mahrer PR, Azen SP, Detrano R, Selhub J, Alaupovic P, Liu C, Liu C, Hwang J, Wilcox AG, Selzer RH, for the BVAIT Research Group. High-Dose B Vitamin Supplementation and Progression of Subclinical Atherosclerosis: A randomized controlled trial. Stroke 2009; 40: 730-36.

[7] Verhoef P, Stampfer MJ, Buring JE, Gaziano JM, Allen RH, Stabler SP. Homocysteine metabolism and risk of myocardial infarction: relation with vitamins B6, B12 and folate. Am J Epidemiol 1996; 143: 845-59.

[8] Bonaa KH, Njolstad I, Ueland PM, Schirmer H, Tverdal A, Steigen T, Wang H, Nordrehaug JE, Arnesen E, Rasmussen K, the NORVIT Trial Investigators. Homocysteine Lowering and Cardiovascular Events after Acute Myocardial Infarction. NEJM 2006; 354: 1578-88.

[9] Nishtar S. The role of vitamins as risk modifying agents in coronary artery disease. Pak J Cardiol 1999; 10: 5-7.

[10] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry1972; 18: 499-502.

[11] Rothenberg SP, daCosta M, Rosenberg Z. A radioassay for serum folate: use of a two-phase sequential incubation, ligand-binding system. N Engl J Med 1972; 286:1335-ss39.

[12] Rothenberg SP. Application of competitive ligand binding for the radioassay of vitamin B12 and folic acid. Metabolism 1973; 22: 1075-82.

[13] Lui A, Lumeng L, Aronoff GR, Li TK. Relationship between body store of vitamin B6 and plasma pyridoxal-P clearance: metabolic balance studies in humans. J Lab Clin Med 1985; 106: 491-97.

[14] Camp VM, Chipponi J, Faraj BA. Radioenzymatic assay for direct measurement of plasma pyridoxal 5′-phosphate. Clin Chem1983; 29: 642-44.

[15] Iqbal MP, Khan AH, Yousuf F. A simple and sensitive radioenzymatic assay for pyridoxal-5′-phosphate in human plasma. Pak J Zool 2003; 35: 231-35.

[16] Hansen CM, Shultz TD, Kwak HK, Memon HS, Leklem JE. Assessment of vitamin B-6 status in young women consuming a controlled diet containing four levels of vitamin B-6 provides an estimated average requirement and recommended dietary allowance. J Nutr 2001; 131: 1777-86.

[17] Bates CJ, Mansoor MA, van der Pols J, Prentice A, Cole TJ, Finch S. Plasma total homocysteine in a representative sample of 972 British men and women aged 65 and over. Eur J Clin Nutr 1997; 51:691-97.

[18] Chou ST, Ko LE, Lim PS, Huang JL, Yang CS. Effect of age and sex on plasma total homocysteine in Taiwanese subjects. Chin J Physiol 2000; 43:159-64.

[19] Mansoor MA, Bergmark C, Svardal AM, Lonning PE, Ueland PM. Redox status and protein binding of plasma homocysteine and

other aminothiols in patients with early-onset peripheral vascular disease, Homocysteine and peripheral vascular disease. Arterioscler Thromb Vasc Biol 1995; 15: 232-40

[20] Subar AF, Harlan LC, Mattson ME. Food and nutrient intake differences between smokers and non-smokers in the US. Am. J Public Health 1990; 80:1323-29.

[21] Nygard O, Vollset SE, Refsum H, Stensvold I, Tverdal A, Nordrehaug JE. Total plasma homocysteine and cardiovascular risk profile. The hordaland homocysteine study, JAMA 1995; 274:1526-33.