Original Article

Plasma homocysteine level in relation to folate and vitamin B6 status in apparently normal men

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The prevalence of subclinical deficiencies of folate and vitamin B_6 in India is high and preliminary investigation showed higher mean plasma total homocysteine level in Indians compared with the values reported for western populations. The present study was carried out in 40 apparently normal men to examine the relationship between plasma total homocysteine level and folate and vitamin B6 status. The mean plasma homocysteine level was high and was inversely related to folate status as judged by red blood cells or plasma folate concentrations. There was no significant relationship between fasting homocysteine level and vitamin B6 status.

Key words: India, plasma folate, plasma homocysteine, red blood cell folate, vitamin B6 status.

Introduction

The prevalence of coronary artery disease (CAD) is reported to be high among migrant Asian Indians and people within the Indian subcontinent.[1–4] Extensive studies in western populations demonstrate that elevated plasma lipid levels, increased blood pressure, abdominal obesity and smoking habit are associated with increased morbidity and mortality from CAD.[5] However, these conventional risk factors of CAD do not explain all the mortality and morbidity due to CAD in the Indian population.[6,7] Several recent studies have implicated elevated plasma levels of homocysteine (Hcy) as an independent risk factor for CAD.[8] An earlier study carried out in India in 35 cases with angiographically defined CAD lesions did not show a significant difference in plasma Hcy levels compared with the values in matched controls.[9] However, the mean plasma Hcy levels in both groups were very high compared with the normal range of values reported in the western population.[8,10] Deficiencies of folate, vitamins B12 and B6 are known to raise plasma Hcy concentration and prevalence of subclinical deficiencies of folate and vitamin B6 are high in India.[11]

The present study was carried out in apparently normal men to examine the relationship of plasma Hcy level with folate and vitamin B6 status.

Subjects and methods

Forty apparently normal male staff members of the National Institute of Nutrition in the age range of 38–58 years were subjects for the present study and it was approved by the medical ethical committee of the Institute. The subjects were from middle- and high-income groups. Heights and weights were recorded and body mass index (BMI) was calculated. Fasting blood samples were collected using ethylenediamine tetraacetic acid (EDTA) as an anticoagulant and were immediately kept on ice. Plasma and cells were separated within 1h of obtaining the blood samples and the samples were stored at –20°C.

The plasma Hcy levels were measured according to the method of Ubbink *et al.*[12] Briefly, to measure the total Hcy content (i.e. sum of free Hcy and disulfide-bound Hcy) the plasma samples were reduced with tri-*n*-butylphosphine (Sigma, St Louis, MO, USA) prior to protein precipitation. The plasma Hcy was derivatized with ammonium 7-fluorobenzo-2-oxa-1,3diazole-4-sulfonate (SBD-F), obtained from Sigma. The SBD derivative of Hcy was separated on a high-performance liquid chromatography column (Supelcosil LC-18-DB, Supelco, Bellefonte, USA) and the fluorescence intensity was measured with excitation at 385 nm and emission at 515 nm. The detector signal was recorded and the peak area calculated by an integrator.

Plasma and red blood cells (RBC) folate levels were measured microbiologically using *Lactobacillus casei* as the test organism. Erythrocyte aspartate aminotransferase activation coefficient (EAAT-AC) was determined as an index of vitamin B6 status.[13] Information on the frequency and quantity of intake of vitamin B12-containing foods was collected by interviewing the subjects. Dietary intake of vitamin B12 was calculated based on the values given in Nutritive Value of Indian Foods.[14]

Apart from means and standard errors, Pearson's product moment correlation coefficient was calculated to establish the relationship between plasma Hcy level and RBC or plasma folate concentration. The cut-off level used for RBC folate is 315 nmol/L and that for plasma folate is 6.8 nmol/L.[15] Unpaired *t*-test was used to compare the mean plasma Hcy values of subjects whose folate status was adequate with the

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mean value of subjects who had biochemical evidence of folate deficiency.

Results

Although mean plasma and RBC folate levels were above the cut-off limit of 6.8 nmol/L and 315 nmol/L, respectively (Table 1), 42.5% of the subjects had subclinical folate deficiency as judged by RBC folate level < 315 nmol/L (Table 2). The mean plasma Hcy values were significantly lower in the subjects whose folate status was adequate as judged by the RBC ($P < 0.001$) or plasma ($P < 0.05$) folate levels compared with the mean values of subjects who were biochemically deficient in folate (Table 2). There was a significant ($P < 0.01$) inverse correlation between plasma Hcy level and RBC or plasma folate concentration (Table 2).

The relationship between plasma Hcy level and vitamin B6 status was not significant although 35% of the subjects had biochemical deficiency of vitamin B6 as judged by EAAT-AC > 1.8.

The semiquantitative assessment of the dietary intake of vitamin B12-rich foods by the subjects revealed that 35% of the subjects were vegetarians and the rest were omnivores. Milk and curds were the only dietary sources of vitamin B12 for vegetarians and the intake ranged between 250 mL and 500 mL/day which corresponds to approximately 0.35 µg–0.77 µg dietary intake of vitamin B12 per day. Among the omnivores, 55% took about 200 g of flesh foods (meat or chicken) once a week, one or two eggs per week and 100 mL–300 mL of milk or curds daily. This corresponds to 5.25 µg–7.25 µg of vitamin B12 intake per week. The dietary intake of vitamin B12 of the remaining 10% of the subjects was about 2 µg/day.

Table 1. Plasma homocysteine, folate and vitamin B6 status of the subjects

Parameters	Mean ± SE
Age (years)	43.70 ± 1.08
BMI	23.35 ± 0.58
Plasma	
Total Hcy (µmol/L)	19.83 ± 1.25
Folate (nmol/L)	11.56 ± 1.07
RBC folate (nmol/L)	372.40 ± 26.26
EAAT–AC	1.73 ± 0.02

BMI, body mass index; Hcy, homocysteine; RBC, red blood cells, EAAT-AC, erythrocyte aspartate aminotransferase activation coefficient.

Table 2. Relationship between plasma homocysteine level and folate status

Folate (nmol/L)	No. of subjects	Plasma Hcy (µmol/L) Mean ± SE	Correlation coefficient
RBC			
> 315	23	14.660 ± 0.724***	0.733**
< 315	17	26.830 ± 1.629	
Plasma			
> 6.8	28	18.300 ± 1.460*	0.647**
< 6.8	10	25.070 ± 2.116	

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Discussion

The result of the present study is in agreement with the earlier observation regarding the high prevalence of elevated plasma Hcy levels in Indians.[9] A study in Quebec, Canada in 380 apparently normal men of similar age range to the present study reported a mean (SD) plasma Hcy concentration of 9.7 ± 4.9 µmol/L.[16] This value is significantly lower than the mean (SD) value observed in Indians (19.83 ± 7.91µmol/L). The technique used for the estimation of Hcy in these two studies was similar.

Subclinical folate deficiency is a significant contributing factor for the higher plasma Hcy concentration observed in these apparently normal men. The low RBC folate concentrations suggest that folate deficiency was of longer duration because it takes a 2–3-month period of low dietary folate intake for a reduction in RBC folate level.[17] Kang *et al.* have reported hyperhomocysteinaemia in subjects with low normal levels of serum folate (7–9 nmol/L) and a concentration of more than 10 nmol/L was associated with normal plasma Hcy concentration.[18] Serum folate level can be influenced by immediate dietary intake of folate-rich food.

Vitamin B6 status as assessed by EAAT-AC did not show any significant correlation with fasting plasma Hcy concentration. A study carried out in the South African general population reported that pyridoxine supplementation (10 mg) for a period of 6 weeks to the subjects with moderate hyperhomocysteinaemia did not significantly alter fasting plasma Hcy levels whereas supplementation with folic acid (0.65 mg) for the same period reduced plasma Hcy levels by 42.7%.[19] Pyridoxal phosphate-dependent enzymes are involved in the transulfuration pathway of Hcy metabolism and an increase in plasma Hcy concentration has been reported after methionine loading in subjects with low plasma pyridoxal phosphate concentration, suggesting that biochemical deficiency of vitamin B6 may raise plasma Hcy concentration after a protein-rich meal.[8]

Indians settled in the UK appear to have high mortality from CAD compared with Europeans and Africans.[6,7,20] This difference could not be explained by well-established risk factors of CAD such as high- and low-density lipoprotein cholesterol concentrations, hypertension, smoking habits or fasting blood glucose level. A recent study reported higher plasma Hcy concentration in Indians in the UK compared with age- and sex-matched Europeans and suggested that this may perhaps contribute to the higher mortality from CAD in Indians.[21] However, further studies are required to confirm this observation. Elevated plasma Hcy levels have also been reported in Sri Lankans.[22]

There is not much information on the prevalence of subclinical vitamin B12 deficiency in India. Strict vegetarians may develop vitamin B12 deficiency after a prolonged period as vitamin B12 is present only in animal foods and its consumption is low in India. The daily requirement of 1 µg of vitamin B12 was not achieved by the vegetarians in the present study. Vegetarians have been reported to have higher plasma Hcy concentration than omnivores.[23]

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