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Original Article

Vitamin B12 Deficiency and Hyperhomocysteinemia in Rural and Urban Indians

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Abstract

Background : Low vitamin B12 concentration in South Asian Indians is common, but the exact prevalence is not known.

Aim : To investigate prevalence and associations of low vitamin B12 concentration and hyperhomocysteinemia in rural and urban Indian men living in and around Pune, Maharashtra.

Method : We studied 441 middle-aged men (149 rural, 142 slum and 150 urban middle-class residents, mean age 39 y). Data on lifestyle, socio-economic status, nutrition and medical history were obtained. Circulating concentrations of vitamin B12, folate, ferritin, total homocysteine (tHcy), and haematological indices, and cardiovascular risk variables were measured.

Results : Median plasma B12 concentration was low (110 pmol/L): Overall, 67% of men had low vitamin B12 concentration (<150 pmol/L) and 58% had hyperhomocysteinemia (>15 µmol/L). Of the urban middle class, 81% had low vitamin B12 concentration and 79% had hyperhomocysteinemia. Low vitamin B12 concentration contributed 28% to the risk of hyperhomocysteinemia (population attributable risk) while low red cell folate contributed 2%. Vegetarians had 4.4 times (95%CI 2.1, 9.4) higher risk of low vitamin B12 concentrations and 3.0 times (95%CI 1.4, 6.5) higher risk of hyperhomocysteinemia compared to those who ate non-vegetarian foods frequently. Urban middle-class residence was an additional independent risk factor of hyperhomocysteinemia (odds ratio 7.6 (95%CI 2.5, 22.6), compared to rural men). Low vitamin B12 concentration was related to lower blood haemoglobin concentration and higher mean corpuscular volume, but macrocytic anemia was rare.

Conclusion : Low vitamin B12 concentration and hyperhomocysteinemia are common in Indian men, particularly in vegetarians and urban middle class residents. Further studies are needed to confirm these findings in other parts of India. ©

Introduction

Elevated circulating total homocysteine (tHcy) concentration is a risk factor for cardiovascular disease[1,2] and elevated tHcy or low folate and vitamin B12 concentrations are a risk factor for birth defects, poor pregnancy outcomes and neurocognitive performance.[3-5] Indians in India[6,7] as well as those migrated abroad[8, 9] have high circulating tHcy concentration compared to other ethnic groups. In Caucasian populations not eating folic acid fortified food, hyperhomocysteinemia is usually explained by low blood folate concentrations.[10] In contrast, hyperhomocysteinemia in Indians living in India is more attributable to low concentrations of vitamin B12.[4]

Eventhough low circulating vitamin B12 concentration have been recognised in Indians for a long time,[11] there is little appreciation of this amongst Indian medical professionals and policy makers. This may be due to a number of reasons: 1) vitamin B12 and tHcy are not routinely measured in clinical practice, 2) despite low circulating vitamin B12 concentrations, specific hematological and neurological manifestations consistent with vitamin B12 deficiency are rare, and 3) the majority of previous reports are clinic based and therefore may not represent community prevalence.

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CRISIS (Coronary Risk of Insulin Sensitivity in Indian Subjects) is a community-based study of insulin resistance and cardiovascular risk in rural and urban middle-aged Indian men. We report our findings on vitamin B12 and folate concentration and hyperhomocysteinemia in the CRISIS study.

Research Design And Methods

Study population

Details of design and methods in the CRISIS study have been published.[12] We used multistage random sampling to select 30-50 y old men from 2 villages, 2 slums and 2 middle class areas from in and around Pune. Those with known diabetes mellitus, hypertension, coronary heart disease were excluded. We studied 441 apparently healthy men (149 rural, 142 slum residents and 150 urban middle-class). Participation rates were 86% for rural, 79% for slums and 71% for urban middle-class men.

The study protocol was approved by the Ethical Committee of the King Edward Memorial Hospital and Research Centre and by the local community leaders. Every participant gave a signed consent.

Protocol and data collection

Subjects ate their normal diet and performed their usual activities until the day before the study. Those with acute intercurrent illness (n=15) were rescheduled 4 weeks after recovery. Subjects arrived at the Diabetes Research Unit the night before the study and were then fed a standard local meal, medically examined and an x-ray chest was done. Trained research staff enquired about history of migration, lifestyle factors, nutritional intake and disease symptoms. Only water was allowed overnight.

In the morning, fasting blood samples were collected in EDTA tubes. A 75 g anhydrous glucose tolerance test (WHO 1997) was done. One portion of the whole blood was used for determination of hematological variables, which were measured within 1 hour of blood collection. The remaining blood was immediately centrifuged at 4°C and 2500 g for 15 minutes to obtain EDTA plasma. Aliquots of plasma were stored at –80°C till further analysis.

Standardised anthropometric measurements were made. Blood pressure was measured by an automated machine (UA 767PC, A and D Instruments Ltd, Abingdon, Oxford, UK). Two blood pressure readings were recorded 5 minutes apart after a 15 minutes rest in the supine position: the second reading was used in the analysis.

Self-declared religion was noted. Smoking and alcohol intake were recorded as never, past and current. Education was recorded as completed years of formal education, and monthly income was categorized. Medical history included frequency of upper gastrointestinal (acidity, regurgitation, epigastric pain) and lower gastrointestinal symptoms (diarrhoea, worms, blood and mucus in stools). Use of drugs, including vitamin supplements was noted.

A diet-recall assessed the intake of energy, carbohydrates, proteins and fats on an average day using nutritive values from a local[13] and a national database.[14] A food frequency questionnaire was used to assess the frequency of consumption of foods which were rich in micronutrients and antioxidants. Based on the focus of the present paper, foods rich in vitamin B12, folate and those shown in previous studies to be related to homocysteine were selected and grouped into 5 mutually exclusive groups: milk and milk-containing beverages, coffee, green leafy vegetables, other vegetables and non-vegetarian foods. For each food item, average frequency of consumption over the past year and the normal portion size were ascertained. For some items (vegetables) only seasonal frequency was recorded.

Cardiovascular disease was assessed by the Rose-WHO angina questionnaire[15] and a resting 12 lead electrocardiogram (ECG). Intima media thickness of the common carotid artery (CCA) was measured using ATL UM9 Color Doppler Machine (Philips Bothell, Washington, USA). We validated our intima media thickness measurements against those made by a vascular research laboratory (Prof Michiel Bots, Julius Centre for Health Sciences and Primary Care, University Medical Centre, Utrecht, Netherlands).

Laboratory measurements

Haematological measurements were made on a Beckman Coulter Analyser (A^C.T diff ™, Miami, Florida). Plasma levels of glucose, total and HDL cholesterol, triglycerides, creatinine were measured on a Hitachi 911 automated analyser (Hitachi Ltd, Tokyo, Japan) using standard methods. Plasma insulin was measured using in-house DELFIA method. Insulin resistance was calculated from fasting plasma glucose and insulin concentrations using the homeostasis model assessment (HOMA-R).[16]

Plasminogen Activator Inhibitor-1 (PAI-1) (Hypen Biomed, Andresy, France), C-reactive protein (CRP) (United Biotech Inc. CA, USA) and antibody titres to *Helicobacter pylori* and intrinsic factor were measured using commercial kits. Plasma vitamin B12 and red cell folate were measured using a radioimmunoassay kit (Diagnostic Products Corporation, USA) and plasma total homocysteine (tHcy) was determined using the IMx System (Abbott Laboratories, IL, USA).

Statistical Analysis

Data are presented as median (interquartile range) and percentages for the three residential groups. In the statistical analyses, variables with skewed distribution were log-transformed to satisfy the assumption of normality. Comparison between the three groups was tested using analysis of variance with Bonferroni post-hoc test, or chi square test. Contribution of covariates to differences in 3 populations was assessed by analysis of covariance. Pooled data from the three residential groups was used to test the associations between blood concentrations of vitamin B12, tHcy and folate and other variables. This analysis was either by partial correlations or analysis of variance, adjusting for age and place of residence. In this study, we defined low vitamin B12 concentration as plasma vitamin B12 <150 pmol/L, low folate concentration as red cell folate <283 nmol/L and hyperhomocysteinemia as plasma tHcy >15 μmol/L. We calculated the population-attributable risk for hyperhomocysteinemia of low concentrations of vitamin B12 and folate compared to those who had vitamin B12 and folate concentration above the thresholds. Significant determinants of low vitamin B12 or folate concentrations and hyperhomocysteinemia were investigated by multiple logistic regression analysis. Significant determinants of haemoglobin and mean corpuscular volume count were assessed by multiple linear regression analysis. SPSS version 11.0 for windows (SPSS Inc, Chicago) was used for statistical analysis.

Results

Rural and urban middle-class men had been resident in these places for more than one generation, while slum residents were mostly first generation migrants from villages. The majority of the men were Hindu (91%), others were Muslim (Table 1). Rural men were subsistence farmers and physically very active. Slum residents were daily-wage workers, and were more frequent smokers. Urban middle-class men were more educated, were wealthier and had sedentary jobs; they were taller and heavier than the slum and rural residents.

Circulating vitamin B12, folate and tHcy concentrations and their associations

Distribution and demographic associations

Median plasma vitamin B12 concentration was low in all 3 groups of men, being lowest in the urban middle-class. Using150 pmol/L as threshold, 67% men had low

Table 1 : Demographic and lifestyle variables and cardiovascular risk factors in rural and urban men. The CRISIS study

	Rural (n=149)	Slums (n=142)	Urban middle-class (n=150)
Age (year)	37 (34, 43)	36 (33, 42)	41 (36, 46)
Height (cm)	165 (161, 169)	164 (160, 168)	166 (163, 171) +++
Weight (Kg)	55.6 (50.2, 61.0)	57.2 (49.7, 66.8)	64.8 (57.3, 72.1) ***, +++
BMI (kg/m^2)	20.1 (18.6, 22.2)	21.1 (18.7, 24.7) **	23.6 (21.0, 25.9) ***, +++
Hindu /Muslim (%)	99/1	81/19	93/7
Education e•10th standard (%)	18	24	83 ***, +++
Manual workers (%)	90	75 ***	40 ***, +++
Monthly income (Indian Rupees) (%)			
<1000	53	3	2
1000-5000	45	88	12
5000-10000	2	7	39
≥ 10000	0	2	47
Smoking (Never/Past/Current) (%)	63/13/24	47/14/39 **	48/26/26 *
Alcohol (Never/Past/Current) (%)	71/9/20	35/14/51 **	42/15/43 ***, +++
Gastrointestinal symptoms (Upper/Lower) (%)	18/22	28*/16	38***/10++
H. pylori antibody positive (%)	95	96	82 **, ++
Energy intake (calorie)	2117 (1668, 2599)	2087 (1721, 2738)	2278 (1753, 2741)
Protein intake (g)	56.6 (41.1, 74.8)	58.1 (46.4, 80.4)	62.2 (49.6, 73.9)
Milk drinkers (%)	64	37 ***	55 +++
Coffee drinkers (%)	23	25	55 ***, +++
Vegetables intake (e•alternate day %)	91	99	100
Non-vegetarian food intake (%)			
Never (Lacto-vegetarian)	41	11 *	44 ++
Rare (<1/week)	17	6	15
Medium (e•1/week)	31	40	29
Frequent (e•alternate day)	11	43 *	12 ++
CVD risk factors (%)			
Impaired glucose tolerance	9	11	20***, +
Diabetes mellitus	0	4**	9***
Hypertension (≥ 140/90 mm Hg)	2	4	9 ***, ++
Hypertriglyceridemia (≥ 150 mg/dl)	6	20**	26***
Plasma HDL cholesterol (£ 35 mg/dl)	45	46	51
Plasma creatinine μmol/L	88.4 (79.6, 88.4)	88.4 (79.6, 97.2)	88.4 (79.6, 97.2)

Median (IQR) or percentages. Impaired glucose tolerance and diabetes mellitus defined by WHO 1999 criteria.
*p<0.05, **p<0.01, ***p<0.001, different from rural and adjusted for age. + p<0.05, ++p<0.01, +++p<0.001, different from urban slums and adjusted for age.

vitamin B12-concentration (68% rural, 51% slum residents, 81% urban middle-class). Median red cell folate concentration was in the normal range in the three groups (>283 nmol/L), and low folate concemThere was no significant relationship between plasma vitamin B12 and red cell folate concentrations. Plasma vitamin B12 concentration was inversely related to plasma tHcy concentration (r= -0.41, p<0.001), an association that was independent of red cell folate concentration. Red cell folate concentration was inversely related to plasma tHcy concentration (r= -0.18, p<0.001). Adjusted for age and place of residence, low vitamin B12 concentrations contributed 28.4% to the risk of hyperhomocysteinemia (population attributable risk) while low folate concentrations contributed only 2.2%.

Plasma vitamin B12 concentration decreased and plasma tHcy increased with increasing age (p< 0.01 and p<0.05 respectively). Plasma vitamin B12, tHcy and red cell folate concentrations were not related to body size measurements. Compared to Hindu men, Muslim men had higher plasma vitamin B12 (148 vs 107 pmol/L), lower plasma tHcy (15.8 vs 19.5 µmol/L) and lower red cell folate concentrations (423.7 vs 505.3 nmol/L) (p< 0.01, all), of which difference between plasma vitamin B12 and plasma tHcy became non-significant after adjusting for non-vegetarian food intake. Higher education and income were associated with lower plasma vitamin B12 and higher tHcy concentrations (p<0.05). These relations were not independent of intake of non-vegetarian foods. Smoking and alcohol habits were not related to plasma vitamin B12 or tHcy or to red cell folate concentrations.

Associations with diet and gastrointestinal factors

Only 3 men took vitamin supplements, none containing vitamin B12. Daily energy intakes of these men were lower than those recommended by the Indian Council of Medical Research (2425 kcals/day) in all 3 groups, and were not related to plasma vitamin B12 or tHcy and red cell folate concentrations. Protein intakes were comparable to the ICMR recommendation (60g/day). Higher protein intake was associated with higher red cell folate concentration (p<0.05) independent of age and place of residence.

Food frequency data was available on 424 men. None of these men were vegan. Forty one percent rural, 11% slum residents and 44% urban middle-class men were lacto-vegetarians. Non-vegetarian foods were eaten more frequently by Muslims than Hindus (p<0.001), by those who were less educated or poorer (p<0.001), and by the slum residents compared to urban middle class (p<0.001). The portion size of non-vegetarian foods was usually small (~100 g cooked). Most men ate chicken, fish and eggs and very few ate red meat. There was a progressive and graded relation between frequency of consumption of non-vegetarian foods and plasma vitamin B12 (r= 0.27, p<0.001) and tHcy concentrations (r= -0.26, p<0.001). On univariate analysis (age adjusted) lacto-vegetarians had a 4.3 (95%CI, 2.4, 7.8) times higher risk of low vitamin B12 concentrations and 4.3 (95%CI, 2.4, 7.6) times higher risk of hyperhomocysteinemia compared to those who ate non-vegetarian foods on at least alternate days (data not shown). Ninety-three men had non-vegetarian food frequently (Table 1). Approximately half of the men who ate non-veg food frequently had low vitamin B12 concentration and hyperhomocysteinemia. Nine men had hyperhomocysteinemia despite frequent non-vegetarian food intake, normal circulating vitamin B12, normal folate and plasma creatinine concentrations. Intake of vegetables, milk and coffee was not related to plasma vitamin B12 and tHcy or red cell folate concentrations.

Plasma vitamin B12 and tHcy and red cell folate concentrations were not related to gastrointestinal

Table 2 : Circulating vitamin B12, total homocysteine and folate concentrations and haematological parameters and in rural and urban men. The CRISIS study

	Rural(n=149)	Slums(n=142)	Urban middle-class(n=150)
Plasma vitamin B12 (pmol/L)	119 (73, 171)	145 (90, 241) **	89 (58, 133) ++
<150 pmol/L (%)	68	51 *	81 *, +++
Total Hcy (µmol/L)	14.6 (12.0, 22.6)	14.2 (11.4, 19.7)	23.7 (15.3, 40.7) ***, +++
>15 µmol/L (%)	48	47	79 **, ++
Red cell folate (nmol/L)	522 (424, 647)	461 (360, 585) *	525 (406, 707) ++
<283 nmol/L (%)	7	12	5 ++
Haematological parameters			
Haemoglobin (g/L)	140 (134,147)	143 (137,149)	141 (132, 146)
Anemia (< 135 g/L) (%)	25	19	31
Mean corpuscular volume (fL)	85.7 (82.3, 89.0)	85.1 (81.8, 89.7)	85.3 (82.2,89.3)
Macrocytosis (>100 fL) (%)	1	2	1
Microcytosis (<80 fL) (%)	15	14	15
Thrombocytopenia (<140 * 10^9/L) (%)	4	4	2
Leucopenia (<4.5*10^9/L) (%)	18	5 *	5 *
Plasma ferritin(<45.0 pmol/L) (%)	18	17	21

Median (interquartile range) or percentages. *p<0.05, **p<0.01, ***p<0.001, different from rural and adjusted for age
+ p<0.05, ++p<0.01, +++p<0.001, different from urban slums and adjusted for age.

symptoms, to *H. pylori* antibody titre, or to plasma CRP and PAI-1 concentrations (data not shown). Intrinsic factor antibody results available in 91 randomly selected men, was positive in 3 (2 rural and 1 urban middle-class), none of whom had particularly low plasma vitamin B12 or raised plasma tHcy concentration compared to the rest of the population.

Multivariate associations of low vitamin B12 concentration and hyperhomocysteinemia

In a logistic regression model including age, religion, income, education, place of residence, and food habits, low vitamin B12 concentrations were independently associated only with food habits. Lacto-vegetarians were 4.4 (95%CI 2.1, 9.4) times more likely to have low plasma vitamin B12 concentration compared to men who ate non-vegetarian foods frequently (Table 3).

In a model including age, place of residence, food habits, religion, income, education and plasma creatinine concentration, hyperhomocysteinemia was independently associated with higher plasma creatinine concentrations, urban middle-class residence and lacto-vegetarian food habits. Urban middle-class residents were 7.9 times more likely to have hyper-homocysteinemia compared to rural men (95%CI 2.8, 22.7), and lacto-vegetarians had 4.3 times higher risk compared to those who ate non-vegetarian foods frequently (95%CI 2.1, 8.9), (data not shown). When low vitamin B12 and folate concentration were included as independent variables, both were significantly associated with hyperhomocysteinemia (OR 2.9, 95%CI 1.7, 4.9 and OR 8.4, 95%CI 1.7, 42.8, respectively). This inclusion reduced the strength of association between hyperhomocysteinemia and lacto-vegetarianism (OR 3.0, 95%CI 1.4, 6.5) but not the place of residence (OR 7.6, 95%CI 2.5, 22.6) (Table 3). When we tested this model by including plasma vitamin B12, plasma creatinine, and red cell folate concentrations, as continuous variables the results were similar.

Low folate concentration was not independently associated with any of the following variables: age, place of residence, religion, income, total macronutrient intake and green leafy vegetables intake (data not shown).

Associations with hematological parameters

Plasma vitamin B12 concentration was directly related to blood haemoglobin concentration (r=0.18, p<0.001) and total leucocyte count (r=0.18, p<0.001) and inversely to mean corpuscular volume (MCV) (r= -0.14, <0.01). Red cell folate concentration was inversely related to MCV (r= -0.20, p<0.001). In a multivariate analysis, blood haemoglobin concentration was independently associated with plasma vitamin B12 and ferritin concentrations (partial r= 0.16, and 0.24 respectively, p<0.001, for both) but not to red cell folate concentrations. MCV was independently associated with plasma ferritin (partial r=0.22), and plasma vitamin

Table 3 : Multivariate associations of low vitamin B12 concentration and hyperhomocysteinemia. The CRISIS study

Independent variables	Low plasma vitamin B12 OR (95% CI)	Hyperhomo-cysteinemia OR (95% CI)
Age		
<40 y (n=255)	1	1
≥ 40y (n=186)	1.38 (0.84, 2.26)	1.55 (0.94, 2.54)
Rural (n=149)	1	1
Slums (n=142)	0.65 (0.35, 1.19)	1.89 (0.99, 3.60)
Urban Middle-class (n=150)	1.95 (0.72, 5.25)	7.58 (2.54, 22.61)
Non-veg food		
Frequent (n=93)	1	1
Medium (n=140)	1.81 (1.00, 3.35)	1.75 (0.89, 3.43)
Rare (n=54)	1.98 (0.86, 4.56)	1.47 (0.61, 3.54)
Lactovegetarian (n=137)	4.43 (2.09, 9.40)	3.02 (1.40, 6.50)
Religion		
Hindu (n=392)	1	1
Muslim (n=38)	1.07 (0.45, 2.59)	0.87 (0.33, 2.24)
Monthly Income		
<5000 INR (n=285)	1	1
≥ 5000 INR (n=145)	0.62 (0.24, 1.59)	0.36 (0.12, 1.03)
Education		
< 10^{th} std (n=247)	1	1
≥ 10^{th} std (n=183)	1.26 (0.67, 2.37)	1.43 (0.74, 2.77)
Plasma creatinine	NA	
< 88 µM (n=192)		1
≥ 88 M (n=247)		2.39 (1.44, 3.96)
Vitamin B12	NA	
≥ 150 pmol/L (n=144)		1
< 150 pmol/L (n=293)		2.88 (1.70, 4.89)
Red cell folate	NA	
≥ 283 nmol/L (n=401)		1
< 283 nmol/L (n=34)		8.44 (1.67, 42.77)

*Non-vegetarian food intake adjusted for total energy intake. *Multivariate models included the independent variables that were significantly associated with the dependent variable in univariate analysis.*NA: Not applicable to the model.

B12 (r= -0.13) and red cell folate (r= -0.21) concentrations (p<0.01, for all). One hundred and eleven of these men were anaemic (hemoglobin <135 g/L), 31% of these had microcytosis (MCV<80 fL), but only 2% (n=2) had macrocytosis (MCV>100 fL). Plasma vitamin B12 concentration was low in 65% of those with microcytic anemia, and in 79% of those with normocytic anemia, and in both men with macrocytic anemia. Leucopenia (< $4.5*10^9$/L) and thrombocytopenia (< 140*10/L) were uncommon.

Association with cardiovascular risk

There was a progressive increase in the proportion of men with impaired glucose tolerance, diabetes mellitus, hypertension and hypertriglyceridemia from rural to slum and urban middle class residents. Proportion with low high-density lipoprotein cholesterol and plasma creatinine concentrations were similar in three groups of men.

Plasma vitamin B12, tHcy and red cell folate concentrations were not associated with plasma glucose, total cholesterol, and triglycerides concentrations and blood pressure on continuous analysis. Plasma vitamin B12 was directly ($r= 0.14$, $p<0.05$) and tHcy concentration was inversely ($r= -0.13$, $p<0.05$) related to HDL concentration. Men with hyperglycemia (fasting plasma glucose ≥110 mg/dl or 2h plasma glucose ≥140 mg/dl), hypertension (blood pressure ≥140/90 mmHg) and low HDL cholesterol concentrations (<35 mg/dl) had higher plasma tHcy concentrations compared to their normal counterparts, even after adjusting for age and place of residence. However plasma vitamin B12 and red cell folate concentrations were similar in the two groups of men.

There was no significant relationship between plasma vitamin B12, red cell folate and tHcy concentrations and ECG abnormalities and intima media thickness (data not shown).

Discussion

Our results demonstrate widespread low plasma vitamin B12 concentration in a community based study of rural and urban middle-aged Indian men in Pune, Maharashtra, India, confirming our previous findings of a clinic based study of patients with or without coronary heart disease and diabetes.[5] Low folate concentration was relatively rare. Hyperhomocysteinemia was very common and more ascribable to low vitamin B12 concentration (attributable risk 28%) than to low folate concentration (2%). Lower plasma vitamin B12 concentration was associated not only with higher plasma tHcy concentration but also with lower blood haemoglobin concentration and higher MCV. This suggests that vitamin B12 concentration had metabolic and hematological consequences, although macrocytic anemia was rare, probably because of adequate folate and low ferritin concentration. Low vitamin B12 concentrations were partly explained by vegetarianism. Hyperhomocysteinemia was independently related to vegetarianism and to urban middle-class residence. These findings represent the first community-based study of vitamin B12 and homocysteine status in mainland India.

In the CRISIS study we sampled men from villages, urban slums and urban middle-class to reflect epidemiological, socio-economic and nutritional transition in India. Approximately half the rural and slum residents and 81% of urban middle-class residents had a low vitamin B12 concentration which was partially explained by low dietary intake. A third of all men were lacto-vegetarian and only half of these drank milk regularly, thus excluding important dietary sources of vitamin B12. Vegetarianism was 4 times more common in the urban middle-class (44%) than in the slum residents (11%). Indians living abroad also have low vitamin B12 concentration ascribable to low dietary intake due to vegetarian food habits.[7] One more contributory factor could be impaired food vitamin B12 absorption.[17] Gastrointestinal symptoms and presence of *H. pylori* antibodies were not related to low vitamin B12 concentration in our study. However, this possibility deserves formal investigation. Tropical sprue is also an unlikely cause because it usually causes folate deficiency [18,] which is in contrast to our data. Antibodies to intrinsic factor were present in less than 5% of subjects, suggesting that pernicious anemia is not a common cause. The ultimate source of vitamin B12 in nature is microbes. The association of higher education and income with lower vitamin B12 concentration could reflect a lack of microbial vitamin B12 from ingestion of contaminated food and water, as well as recycled colonic bacteria.[19]

Hyperhomocysteinemia was very common in these men and the median plasma tHcy concentration was twice that reported in White-Caucasian populations.[20,21] The aetiology of hyperhomocysteinemia appears multifactorial, with contributions from diminished renal function (higher plasma creatinine but still within normal range), vitamin B12 deficiency associated with vegetarianism, folate deficiency and urban residence. Contribution of vegetarianism and low vitamin B12 concentration was much more important than that of low folate concentration. Other known determinants of tHcy, such as smoking, coffee drinking and high alcohol intake[20,21] were not associated with hyperhomocysteinemia in this population. Occurrence of hyperhomocysteinemia in a substantial number of men eating non-vegetarian food could either be due to small intake of these foods, rarity of red meat consumption or due to reduced absorption of food vitamin B12. The substantial contribution of urban middle class residence to hyperhomocysteinemia, independent of dietary habits and blood vitamin status remains unexplained and needs further investigation. Urban middle class represent the leading edge of epidemiologic and nutritional transition, and differ from the rural and slum residents in a number of social, economic, behavioural and other lifestyle factors. Some or all of these might combine to increase the risk of hyperhomocysteinemia.

Hyperhomocysteinemia in some men with normal circulating vitamin B12 concentration suggests a contribution of additional mechanisms, for example, associated deficiency of other relevant nutrients such as folate (rare in our population), vitamin B2, vitamin B6 or betaine or its precursors.[22] Another possibility is defective transport into the cells. Migrant Indians in the US had higher plasma tHcy concentrations compared to white Caucasians at equivalent plasma vitamin B12 concentrations. This was only partly explained by low vitamin B6 concentrations.[8]

In addition to hyperhomocysteinemia, low plasma vitamin B12 concentration was associated with lower blood haemoglobin concentration and larger red cell volume (in the 'normal' range), though macrocytic anaemia was rare. This may be due to adequate folate concentration and low iron status (ferritin), both of which prevent macrocytosis. In clinical practice lack of 'macrocytosis' is usually interpreted as indicating normal vitamin B12 status, this may have resulted in underdiagnosis of low vitamin B12 status in India. A study in migrant Indians in the UK[23] as well as a recent study in Delhi[24] reported that dietary deficiency of vitamin B12 was a more common cause of megaloblastic anaemia than was pernicious anaemia. We have previously demonstrated high circulating methyl malonic acid concentrations in middle-aged men and women,[5] a specific indication of impaired action of vitamin B12.

Hyperhomocysteinemia was associated with some components of the metabolic syndrome i.e. hyperglycemia, hypertension and low levels of plasma HDL-cholesterol concentration but not with HOMA insulin resistance, inflammatory and prothrombotic markers. It was also not related to intima media thickness or ECG abnormalities in this cross sectional study. A study in migrant Indians in the US showed an association between plasma tHcy concentration and insulin resistance.[8] Recently high plasma tHcy concentration has been shown also to predict incident diabetes (both gestational and post-gestational).[25,26] In Indians as yet no study has demonstrated an association between hyperhomocysteinemia and cardiovascular disease. These associations need to be tested in prospective studies. Studies in Pune have demonstrated an association between high plasma maternal tHcy concentration and small for gestational age babies.[4] In migrant Indians in the UK, vegetarian habits and low vitamin B12 concentration have also been associated with increased risk of tuberculosis.[27]

Thus, low plasma vitamin B12 concentration and hyperhomocysteinemia are common in middle-aged Indian men in Pune, Maharashtra. Despite repeated demonstration of low plasma vitamin B12 concentration in Indians for over 50 years there is poor appreciation of the problem by both the medical profession and the policy makers. Vegetarianism and urban middle class residence are important aetiological factors. Vegetarianism in India is multigenerational, lifelong and based on religious and cultural beliefs. Further research is necessary to improve our understanding in this area and to investigate novel ways to treat and prevent the problem.

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