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# Hyperhomocysteinemia, and low intakes of folic acid and vitamin B12 in urban North India

**Summary** *Background and Aim* An adverse coronary risk profile has been reported amongst rural-to-urban migrant population living in urban slums undergoing stressful socio-economic transition. These individuals are likely to have low intakes of folic acid and vitamin B12, which may have an adverse impact on serum levels of homocysteine (Hcy). To test this hypothesis, we studied serum levels of Hcy in subjects living in an urban slum of North India and healthy subjects from urban non-slum area. *Methods* Group I consisted of 46 subjects (22 males and 24 females) living in an urban slum, while group II consisted of healthy subjects (n = 26, 13 males and 13 females) living in the adjacent non-slum area. Anthropometric measurements, biochemical profile (fasting blood glucose, total cholesterol, serum triglycerides, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol) and fasting serum levels of Hcy were measured. Dietary intakes of folic acid, vitamin B12, vitamin B1, and iron were calculated by the 24-hour dietary recall method. Serum levels of Hcy were correlated with dietary intakes of nutrients, anthropometry, and metabolic variables. *Results* Sex-adjusted serum levels of Hcy in μmol/L (Mean ± SD) were high, though statistically comparable, in both the groups (group I: 20.8 ± 5.9 and group II: 23.2 ± 5.9). Overall, higher than normal serum levels of Hcy (> 15 μmol/L) were recorded in 84 % of the subjects. A substantial proportion of subjects in both groups had daily nutrient intakes below that recommended for the Asian Indian population (folic acid: 93.4 % in group I and 96.7 % in group II, vitamin B12: 76.1 % in group I and 88.4 % in group II). However, between the two groups, average daily dietary intakes of both the nutrients were statistically comparable. As compared to non-vegetarians, vegetarians showed lower intakes of folic acid ($p < 0.01$) and vitamin B12 ($p < 0.01$) in both groups. On multivariate linear regression analysis with serum Hcy as the response variable and vegetarian/non-vegetarian status and sex (male/female) as predictor variables, higher serum levels of Hcy were observed in vegetarians vs non-vegetarians ($\beta = 4.6$, $p < 0.05$) and males vs females ($\beta = 5.3$, $p < 0.01$). *Conclusions* Low intakes of folic acid and vitamin B12, and hyperhomocysteinemia, in both the healthy population living in urban slums and adjacent urban non-slum areas, are important observations for the prevention of nutritional and cardiovascular diseases in the Indian subcontinent.

**Key words** Homocysteine – Vegetarians – Coronary heart disease – Asian Indians – Folic acid – Vitamin B12

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## Introduction

Cardiovascular diseases are a major health problem amongst Asian Indians [1, 2]. Studies on native and immigrant Asian Indians indicate increased susceptibility for accelerated and premature coronary heart disease (CHD) [3] and consequently a higher mortality as compared to other ethnic groups [4]. The higher prevalence of abdominal obesity, insulin resistance and dyslipidemia are largely responsible for this phenomenon [5–7]; however, in many instances, no conventional risk factor could be demonstrated in those affected. The role of non-conventional risk factors in atherosclerotic complications in Asian Indians, thus, remains to be investigated.

Recent studies suggest elevated serum level of homocysteine (Hcy), a sulphur containing amino acid, as a possible risk factor for CHD [8]. Patients with homocysteinuria, an autosomal recessive condition [9, 10], and other genetic defects of enzymes that result in high serum levels of Hcy, suffer from premature atherosclerosis (peripheral vascular disease, stroke, and myocardial infarction) [11–14]. Several prospective studies have shown a positive association of hyperhomocysteinemia with CHD [11]. Vitamins B12, B6 and folic acid are essential to the metabolism of Hcy [15] and supplementation of these vitamins, particularly folic acid, decreases levels of plasma Hcy [15, 16]. Such dietary interventions hold promise for the prevention of CHD [17]. In developed countries, the prevalence of hyperhomocysteinemia is 5% in the general population, and 13–47% in patients with vascular diseases [9].

Differences in the genetic, dietary, and lifestyle profile restrict the generalization of results from other ethnic groups to the Asian Indians. In a large population-based study, the plasma level of Hcy was reported to be an independent risk factor for CHD in Asian Indians in the UK (predominantly of north Indian descent) [18] and hyperhomocysteinemia was attributed to low serum levels of folic acid and vitamin B12. Another study on immigrant Asian Indians also corroborates these observations [19]. Of note, data from Asian Indians in India are mostly anecdotal. Studies conducted in southwest and southeast provinces of India indicate no relationship of plasma levels of Hcy with CHD [20, 21]. On this aspect there is no study on Asian Indians residing in the northern part of India.

Being relatively affluent, the diet and lifestyle practices of immigrant Indians are different as compared to those in India. Different geographical regions of India have different dietary habits influenced by caste, religion and socio-economic status. The high prevalence of conventional atherogenic risk factors, namely dyslipidemia, glucose intolerance, obesity, hypertension, and smoking has been recorded in people belonging to low socio-economic strata living in urban slums recently [22]. In addition, imbalanced nutrition (high saturated fat intake, low intakes of fiber and anti-oxidants) is prevalent in such population [23]. Furthermore, serum levels of folic acid and vitamin B12 are deficient in Asian Indians as shown by a study from a Western Indian State [24]. In particular, hyperhomocysteinemia has not been investigated previously in economically deprived Asian Indians living in urban slums. Therefore, it is important to investigate the prevalence of hyperhomocysteinemia, and its relationship with folic acid and vitamin B12 in this population group. Theoretically, in the background of a high prevalence of other coronary risk factors, hyperhomocysteinemia would further contribute to the acceleration of atherosclerosis.

We, therefore, hypothesize that the rural-to-urban migrant population residing in urban slums would have high prevalence of hyperhomocysteinemia due to poor nutritional state, in particular, due to the deficiencies of folic acid and vitamin B12. To test this hypothesis we studied serum levels of Hcy in subjects living in an urban slum and correlated it with dietary intakes of folic acid, vitamin B12, vitamin B1, iron, anthropometry and metabolic variables. The data were compared with healthy subjects living in the adjacent urban non-slum area.

## Materials and methods

The study sample consisted of 72 subjects belonging to two different groups. Group I (cases) consisted of asymptomatic subjects (n = 46, 22 males and 24 females) living in an urban slum in the southern area of New Delhi. Group II consisted of subjects (n = 26, 13 males and 13 females) who volunteered to participate in the study in response to a local advertisement. This group consisted of apparently healthy subjects with presumably better nutrition intake, living in adjacent urban non-slum area. The subjects in group I were drawn from a previously carried out population-based study, Delhi Urban Slum Project (DUSP) (n = 532, 362 females and 170 males). The details of recruitment, epidemiological methods, and field procedures are given elsewhere [22]. Briefly, an urban slum colony *Gautam Nagar* consisting of a population of approximately 30,000 people living in a sixteen square kilometer area was selected for the survey. Using the electoral list from the Slum Development Wing, Government of India, the area was divided into four equal sectors for the purpose of survey. The population living in this area consisted of people migrated from the states adjoining New Delhi. Monthly income of more than 90% of the people was < 4000 rupees (~US $ 70). Pre-survey sensitization camps were organized for the population by the medical team. The field interviews and other procedures were completed in 20 months, starting January 1998.

The institutional ethics committee, consisting of ten members, inclusive of the faculty members, the Dean, legal advisors, representative of Indian Consul of Medical Research, and refered professors of Medicine, approved the study. Further, all the other procedures as applicable to clinical protocols carried out in the institution were strictly followed

A workshop was held on standardizing the techniques of anthropometric measurements for the field personnel, tested on 50 subjects as a pre-project pilot study. Two medical teams, which included physicians and dieticians, administered a detailed questionnaire incorporating demographic profile, social-economic aspects, migration pattern, relevant symptoms, tobacco and alcohol consumption, and physical activity profile. A detailed food frequency questionnaire was also administered. Subjects with manifest CHD, history of cerebrovascular accident, peripheral vascular disease, severe end-organ dysfunction, acute illness, acquired immunodeficiency syndrome, pregnancy, and patients with history of excessive alcohol consumption and substance abuse were excluded from the study. An informed written consent was obtained from the study participants before the clinical examination and laboratory investigations were performed. Physical examination including anthropometry was performed on all the subjects at the study site. Blood pressure was recorded in the sitting position with a standard mercury sphygmomanometer.

Subjects in group II were admitted for a short period in the General Clinical Research Center at the hospital. In order to avoid any possible bias due to inter-observer variation, the same two physicians and dieticians recorded information on clinical and nutrient profile from subjects in both groups.

## Assessment of nutrient intake

Nutrient information was analyzed using a pre-tested 24-hour food recall proforma prepared by the investigating team according to the guidelines laid down by National Institute of Nutrition, Hyderabad, India [25]. The proforma was in two languages, Hindi and English, and consisted of three sections. First section dealt with the 24-hour nutrient intake, listing the details of morning tea, breakfast, mid-morning, lunch, evening tea, dinner and bedtime snacks. In the second section, unusually consumed food items were recorded. The consumption was recorded on weekly, bi-weekly and monthly basis. The third and last section was designed to collect the information regarding the type and amount of fat used for the daily cooking. Standard sets of common utensils utilized in Indian households were used to assess the portions of food articles. Daily intake of nutrients (vitamins B1, B12, folic acid, iron) was calculated by adding all the raw foods consumed on daily, weekly, bi-weekly and monthly basis. Data analysis of the dietary parameters was carried out using software developed by the investigating team using the standard nutrient values of Indian foods [26]. The software was previously used in evaluating nutritional data in a recently published study [23].

## Anthropometric measurements

Body weight (to the nearest 0.1 kg) and height (to the nearest 0.1 cm) were recorded without shoes while allowing only light clothes. BMI was calculated by using the formula weight/height$^2$ [kg/m$^2$]. Waist circumference (WC) was measured midway between the iliac crest and lowermost margin of the ribs and hip circumference was measured at the maximum circumference of buttocks. The mean of three readings of each measurement was taken for the calculation of the waist-hip ratio (W-HR). Biceps, triceps, subscapular and suprailiac skinfolds were measured using Lange skinfolds calipers (Beta Technology Inc., Santa Cruz, CA, USA) to the nearest 1 mm. The mean of three readings was recorded at each site. The biceps fat pad was measured at the level of the nipple line, and triceps skinfold triceps fat pad was measured midway between the acromion process of scapula and the olecranon process. Fat pads at the inferior angle of the scapula and superiorly on the iliac crest directly in the mid-axillary line were measured for subscapular and suprailiac skinfolds. A standard equation was used for the calculation of percentage body fat (%BF) from the sum of four skinfolds (sigma 4 SF) [27]. The equation has been validated in the Asian Indians for the calculation of % BF [28].

## Biochemical samples and analysis

A venous blood sample was obtained after a 12-hour overnight fast for the estimation of blood glucose, lipids, and serum levels of Hcy. Estimation of total cholesterol (TC), serum triglycerides (TG), and high-density lipoprotein cholesterol (HDL-c) was performed on the fasting venous blood sample using commercial kits (Randox Laboratories Ltd., UK). The value of LDL-c was calculated using Friedwald's equation [29].

Blood samples were transported on ice and immediately processed for serum separation. Ten milliliters of venous blood was drawn in a plain glass tube and allowed to clot for 30 minutes. After clot formation, it was gently broken using a glass rod and serum was separated by low speed centrifugation (2000 rpm for 20 min). The separated serum was frozen and stored at – 70 °C.

Serum levels of Hcy were estimated using a commercial ELISA kit (Axis Homocysteine EIA, Axis Shield AS,

Norway). All the serum samples were thawed to room temperature before the assay. Total Hcy concentration was determined after protein-bound Hcy was converted to free Hcy, which was then enzymatically converted to S-adenosyl-L-Hcy (SAH) in a separate procedure by S-adenosyl-L Hcy hydrolase. This solid phase enzyme immunoassay was based upon competition between SAH in the sample and immobilized SAH bound to the walls of microtiter plates for binding sites on monoclonal antibody anti-SAH antibodies. After removal of anti-SAH antibodies not bound to plates, a secondary rabbit anti-mouse antibody labelled with enzyme horse-radish peroxidase (HRP) was added. The HRP activity was measured using a ELISA reader (Bio Rad, USA) by estimating the absorbance after addition of the substrate. Concentrations of Hcy in samples were calculated from the standard curve. All the samples were assessed in a single assay. As per the specifications of the manufacturer the calibrator range of the assay was 2.2 to 50 μmol/L. The mentioned intra-assay precision using three levels of controls – low, medium and high – was 7.3 %, 6.8 % and 5.2 % respectively. The total precision was 9.3 %, 8.1 % and 7.1 % for the three respective levels of controls. Using duplicate samples the intra-assay variation was in the range of 1.3–3.8 % (mean 2.14 %).

### Definitions

Obesity was defined as BMI > 25 kg/m$^2$ [30]. % BF was defined as indicative of obesity if it was > 25 % in males, and > 30 % in females [31]. Normal fasting Hcy was defined as 5–15 μmol/l [32–34]. For statistical purposes, fasting levels of serum Hcy (μmol/L) were divided into tertiles, tertile 1: 5–15, tertile 2: 16–30, and tertile 3: > 30. The recommended daily allowances (RDA) of various dietary nutrients in Asian Indians were defined as per the National guidelines: vitamin B12 – 1 μg/day and folic acid – 100 μg/day (both males and females), vitamin B1 – 1.2 μg/day (males) and 0.9 μg/day (females), and iron – 28 mg/day (males) and 30 mg/day (females) [26]. A subject who never ate meat, poultry, fish, or eggs was defined as a vegetarian, otherwise considered as a non-vegetarian.

## Statistical methods

Data were recorded on a pre-designed performa and managed on an Excel spreadsheet. All the entries were checked for any error. Descriptive statistics for quantitative variables were computed by mean and standard deviation. Means in the two groups were compared by Student's t test/Wilcoxon Rank Sum test as appropriate. Pearson's/Spearman's rank correlation coefficient was computed to assess the extent of correlation among two quantitative variables. Analysis of co-variance (ANCOVA) was used to compute mean ± SD of anthropometric, biochemical and dietary variables adjusted for sex. These adjusted values were subsequently compared between the groups. Multivariate linear regression analysis was performed to determine the effect of diet (vegetarian/non-vegetarian) and gender. STATA 7.0 Intercooled Version (STATA Corp, Houston, Texas, USA) was used for data analysis. In this study, $p < 0.05$ has been considered as statistically significant.

## Observations

### Demographic profile

The mean ± SD age of the subjects in the two groups (group I: 24.7 ± 3.8 years; group II: 23.0 ± 2.9 years) was statistically comparable. In group I, 22.7 % males were smokers as compared to none in group II. A greater number of males consumed alcohol in group I (36.4 %) as compared to group II (15.4 %) ($p = 0.18$). No females in either group smoked or consumed alcohol. The proportion of vegetarians and non-vegetarians was almost equal in group II (53.8 % and 46.2 %, respectively). In group I, the proportion of non-vegetarians was significantly higher (84.8 %) than vegetarians (15.2 %) ($p = 0.001$).

### Anthropometric and body fat profile (Table 1)

Mean values of BMI and W-HR were comparable between the two groups. However, %BF was observed to be higher in group II as compared to group I ($p = 0.04$). Significantly higher values of biceps skinfolds ($p = 0.007$), subscapular skinfolds ($p = 0.02$), and sigma 4SF ($p = 0.04$) were observed in group II as compared to group I. In group II, W-HR was significantly higher in males as compared to females ($p = 0.002$). Values of all the individual skinfolds were higher in females in both the groups as compared to males. Using BMI to define obesity, two (4.4 %), and one (3.9 %) subjects were observed to be obese, while high % BF was observed in 10 (21.7 %) and 8 (30.8 %) subjects in group I and II, respectively.

### Biochemical profile including serum Hcy levels (Table 2)

A trend towards higher mean level of fasting blood glucose was observed in group I as compared to group II. None of the study subjects were diabetic. Mean values of TC, TG, LDL-c and HDL-c were comparable between the two groups. Females had significantly higher LDL-c in

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**Table 1** Anthropometric profile (Mean ± SD) adjusted for gender using analysis of co-variance

| Anthropometric measurements | Group I (n = 46) | Group II (n = 26) |
|---|---|---|
| Body mass index (kg/m$^2$) | 20.0±3.4 | 21.1±3.4 |
| Waist-hip ratio | 0.82±0.05 | 0.81±0.06 |
| Skinfolds (mm) | | |
| Biceps | 5.8±3.4 | 8.1±3.4[b] |
| Triceps | 11.7±6.0 | 14.5±6.0 |
| Subscapular | 14.3±7.6 | 18.5±7.6[a] |
| Suprailiac | 14.9±7.8 | 16.6±7.9 |
| Sigma 4SF* | 46.7±22.0 | 57.9±22.2[a] |
| Percentage body fat | 21.4±5.8 | 24.3±5.9[a] |

*Group I* Healthy subjects living in urban slum; *Group II* Healthy subjects living in an adjacent urban non-slum area.
* Sum of four skinfolds
[a] $p < 0.05$; [b] $p < 0.01$ as compared to group I

**Table 2** Biochemical and nutrient intake profile of subjects adjusted for gender using analysis of co-variance

| Variable | Group I (n = 46) | Group II (n = 26) |
|---|---|---|
| Biochemical variables (mmol/L) | | |
| Fasting blood glucose | 86.4±13.2 | 80.0±13.3 |
| Total cholesterol | 174.5±39.5 | 158.9±39.8 |
| Serum triglycerides | 113.1±38.9 | 100.7±39.2 |
| Low-density lipoprotein cholesterol | 115.8±38.9 | 99.1±39.1 |
| High-density lipoprotein cholesterol | 41.3±7.0 | 41.3±7.1 |
| Serum homocysteine (µmol/L) | 20.8±5.9 | 23.2±5.9 |
| Nutritional variables (daily dietary intake in µg/d) | | |
| Folic acid | 64.0±19.7 | 62.2±19.9 |
| Vitamin B12 | 0.75±0.62 | 0.55±0.62 |
| Vitamin B1 | 1.3±0.43 | 1.16±0.43 |
| Iron (mg/d) | 17.5±5.0 | 15.8±5.0 |

*Group I* Healthy subjects living in urban slum; *Group II* Healthy subjects living in an adjacent urban non-slum area.
Values in the two groups are statistically comparable

group I ($p = 0.03$) and significantly lower HDL-c in group II ($p = 0.004$) as compared to the males.

Mean serum levels of Hcy showed a trend to be higher in group II as compared to group I. Stratified serum levels of Hcy were as follows: tertile 1 – group I, 13% (males 4.6%, females 20.8%) and group II, 19.2% (males 7.7%, females 30.8%); tertile 2 – group I, 69.6% (males 63.6%, females 75%) and group II, 61.6% (males 76.9%, females 46.1%); and tertile 3 – group I, 17.4% (31.8%, females 4.2%) and group II, 19.2% (males 15.4%, females 23.1%) (Fig. 1). Overall, higher than normal serum levels of Hcy (> 15 µmol/L) were recorded in 84% of the subjects. The proportion of subjects in each tertile was statistically not different between the two groups.

## Profile of nutrient intake (Table 2)

The average daily dietary intakes of folic acid, vitamin B12, vitamin B1 and iron were comparable between the two groups. Males had higher intake of folic acid ($p = 0.001$) and vitamin B12 ($p = 0.006$) in group I and folic acid ($p = 0.001$) and iron ($p = 0.004$) in group II as compared to females. Intakes of vitamin B1 showed a tendency to be higher in males in both groups.

A striking proportion of subjects in both groups had daily dietary intake of various nutrients below the RDA values defined for the Asian Indian population. The most notable deficiencies were: folic acid (93.4% in group I, 96.7% in group II), vitamin B12 (76.1% in group I, 88.4% in group II), and iron (95.6% group I, 100% in group II) (Fig. 1). Vitamin B1 deficiency was observed to a lesser extent (32.6% in group I, 26.9% in group II) (Fig. 1). As compared to non-vegetarians, vegetarians in both groups consistently showed lower intakes of folic acid [group I: 57.2 ± 19.2 µg/day (NS), group II: 46.8 ± 17.6 µg/day ($p = 0.03$)] and vitamin B12 [group I: 0.38 ± 0.21 µg/day ($p = 0.01$), group II: 0.21 ± 0.22 µg/day ($p = 0.04$)].

## Correlations

Significant correlations were observed among intakes of folic acid and vitamin B12 in both vegetarians ($P = 0.68$, $p < 0.001$) and non-vegetarians ($P = 0.48$, $p < 0.001$) (Fig. 2). On multivariate linear regression analysis with serum levels of Hcy as a response variable and vegetarian/non-vegetarian status and sex (male/female) as predictor variables, higher serum levels of Hcy were observed in vegetarians vs non-vegetarians ($\beta = 4.6$, $p < 0.05$) and males vs females ($\beta = 5.3$, $p < 0.01$).

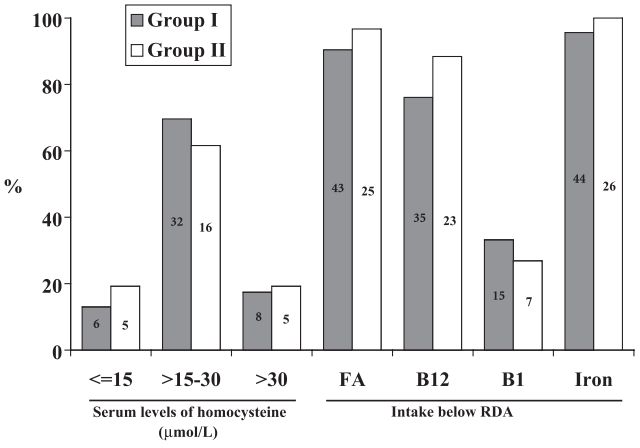


**Fig. 1** Serum levels of homocysteine and low nutrient intake. Group I: Healthy subjects living in urban slum; Group II: Healthy subjects living in an adjacent urban non-slum area; *FA* folic acid; *B12* vitamin B12; *B1* vitamin B1; *RDA* Recommended dietary allowance for Asian Indians [26].

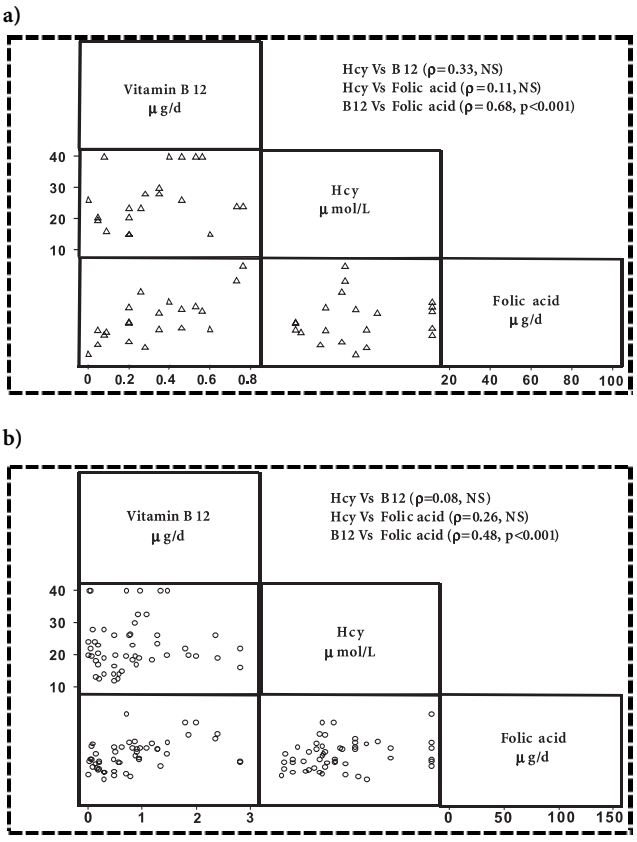


**Fig. 2** Dot plot matrix showing relationship among serum levels of homocysteine, and dietary intakes of vitamin B12 and folic acid in vegetarians (**a**) and non-vegetarians (**b**); *Hcy* Serum homocysteine; *NS* not significant.

## Discussion

Markedly decreased intakes of folic acid and vitamin B12, and striking hyperhomocysteinemia of similar proportion in the urban population despite belonging to different socio-economic classes are important observations in the study. Of particular note, only approximately 16 % of the people had normal levels of serum Hcy. This finding is similar to the observations of Refsum et al., who recorded normal levels of Hcy in approximately 23 % of subjects from a Western province of India [24]. The cut-off value for the definition of hyperhomocysteinemia is the same in the two studies. However, in the Refsum study, the sample was heterogeneous consisting of subjects with CHD, type 2 diabetes mellitus and healthy controls. In the present study only healthy subjects have been included. In contrast, a low prevalence of hyperhomocysteinemia has been recorded in Southern India, 10.7 % in CHD patients and 5.7 % in healthy controls [20] (Table 3). The prevalence rate of hyperhomocysteinemia in another Asian population group, Bangladeshis, residing in East London was 33 % as compared to 15 % in Caucasians [35] (Table 3).

Of significance, the mean level of Hcy in the present study (23.9 µmol/L) is the highest recorded in India; higher than those observed in healthy controls in South India (12.4 ± 3.4 µmol/L [21] and 9.41 ± 3.6 µmol/L [20]), and CHD patients (12.6 ± 4.6 µmol/L [21], 10.9 ± 9.0 µmol/L [20]) (Table 3). In general, high mean levels of Hcy appear to be a common observation in native Asian Indians. Similar results were obtained in Western India (19.7 µmol/L (healthy controls) and 20.0 µmol/L (CHD patients) [24]) and in South-east India (19.7 ± 1.87 µmol/L (healthy controls) and 21.5 ± 2.33 µmol/L (CHD patients) [36]) (Table 3). In another Asian ethnic group (Thai population), high mean levels of serum Hcy were recorded in the control subjects (19.69 ± 8.51 µmol/L) and CHD patients (23.83 ± 11.29 µmol/L) [37] (Table 3).

Several factors (methodology of assay, definition of normal values, age, etc.) may explain markedly different mean levels of serum Hcy and variations in the prevalence rates of hyperhomocysteinemia in Asian Indians and other ethnic groups (Table 3). As compared to the present study, other Indian authors have used different cut-off levels to define normal levels of Hcy based on the 95$^{th}$ percentile of the serum levels of Hcy in the control population, e. g., 15.62 nmol/ml [20] and 17.1 µmol/L [21]. These cut-off levels are not appreciably different from that used by us, and should not be a cause of major discrepancy in the prevalence of hyperhomocysteinemia. However, mean age of the subjects, which may also cause variations in the levels of Hcy, is significantly different in the various studies. Of significance, subjects in all other studies were older as compared to the subjects in the current study, where mean age of the subjects was approximately 24 years. A possible explanation is that older patients with CHD and age-matched controls were recruited in the other studies. For example, the mean age of Thai subjects, where equally high mean levels of Hcy were observed, was significantly higher, 58–60 ± 10 years [37]. Further, a notable feature of the subjects in group I of the current study was their poor economic condition. However, it is difficult to compare the data with those of other studies, since the information of the economic condition of the subjects is not generally available. In UK, 43 % of cases and 39 % of controls belonged to the 'non-manual' class, and hyperhomocysteinemia was not correlated to social class in Asian Indians [18]. In an otherwise elegantly performed study, Refsum et al. fail to mention any correlation of social or economic class of recruited subjects to the plasma levels of Hcy [24].

Notably, most cross-sectional and case-control studies in Asian Indians fail to show any association of hyperhomocysteinemia with CHD [20, 21], except that on Asian Indians in the UK [18]. In Singapore, though marked ethnic differences exist in the prevalence of CHD among Indians, Malays and Chinese, plasma levels of Hcy did not provide any explanation for this phe-

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**Table 3** Inter-ethnic comparative data of serum/plasma levels of homocysteine

| No | Study (reference no) | Population (n) | Mean age (years) | Method | Hcy value (μmol/L) | Hyperhomocysteinemia | |
|---|---|---|---|---|---|---|---|
| | | | | | | cut-off (μmol/L) | prevalence (%) |
| 1 | Hughes et al. 2000 [19] | Asian Indians (250) | Not available | HPLC plasma | M-16.2, F-11.5 | > 14 | M-60, F-21.9 |
| | | Malays (250) | | | M-15.0, F-12.0 | | M-53.9, F-37.8 |
| | | Chinese (250) | | | M-15.3, F-12.2 | | M-56.6, F-30.6 |
| 2 | Leowatanna et al. 2000 [36] | Thai population | | ELISA | | > 17 | |
| | | CHD* (178) | 60.0±10.0 | | 23.85±11.29 | | 62 |
| | | Controls (178) | 58.0±10.0 | | 19.69±8.51 | | 49 |
| 3 | Chambers et al. 2000 [18] | Asian Indians | | HPLC, plasma | | > 12.4 | |
| | | Cases (250) | 52.0±1.3 | | 12.0±4.5 | | 36 |
| | | Controls (500) | 49.0±6.9 | | 10.8±4.0 | | 29 |
| | | Europeans | | | | | |
| | | Cases (300) | 55.3±5.9 | | 11.13.9 | | 27 |
| | | Controls (500) | 49.4±6.5 | | 10.22.9 | | 20 |
| 4 | Chacko et al. 1998 [20] | Asian Indians (South-West) | | HPLC, serum | | > 15.62 | |
| | | CHD (56) | 49.4±12.4 | | 11±9 | | 10.7 |
| | | Controls (53) | 47.9±12.5 | | 9.4±3.6 | | 5.7 |
| 5 | Deepa et al. 2001 [21] | Asian Indians (South-East) | | ELISA, serum | | > 17.1 | |
| | | Controls (18) | 53.0±8.0 | | 12.4±3.4 | | 5.6 |
| | | CHD (21) | 53.0±7.0 | | 12.6±4.6 | | 19.0 |
| | | DM** (18) | 53.0±9.0 | | 10.1±4.4 | | 5.6 |
| | | DM+CHD (20) | 56.0±9.0 | | 10.4±3.9 | | 5.0 |
| 6 | Gheye et al. 1999 [35] | Asian Indians (South-East) | | HPLC, plasma | | > 17 | |
| | | CHD (58) | 52.3±1.2 | | 21.5±2.33 | | 54 |
| | | Controls (58) | 51.6±1.18 | | 19.7±1.87 | | 54 |
| 7 | Vermeulen et al. 2000 [16] | Siblings of patients | | HPLC, plasma | | > 18 | |
| | | Pre-treatment | | | | | |
| | | Vitamin group (78) | 45.8±7.0 | | 14.7±8.2 | | 21.8 |
| | | Placebo group (80) | 46.2±8.1 | | 14.7±8.8 | | 25 |
| | | 2 years post-vitamin therapy | | | | | |
| | | Vitamin group (78) | | | 7.4±1.9 | | |
| | | Placebo group (80) | | | 12.0±5.4 | | |
| 8 | Obeid et al. 1998 [34] | Healthy Bangladeshis (170) | 49.5±10.2 | HPLC, serum | 13.3±4.9 | > 15 | 24 |
| | | Healthy Europeans (43) | 43.8±9.5 | | 8.49±2.16 | | 0 |
| 9 | Refsum et al. 2001 [24] | Western Asian Indians | | HPLC, Plasma | | > 15 | |
| | | Healthy (63) | 44.0 | | 19.7 | | 81 |
| | | Diabetes (41) | 46.0 | | 18.1 | | 76 |
| | | CHD (58) | 48.0 | | 20.0 | | 74 |
| | | CHD + diabetes (42) | 52.0 | | 20.2 | | 79 |
| 10 | Present study (2001) | Asian Indians (North) | | ELISA, serum | | > 15 | |
| | | Subjects in slums*** (46) | 24.7±3.8 | | 23.2±8.4 | | 86.9 |
| | | Controls**** (26) | 23.0±3.0 | | 25.2±8.2 | | 80.7 |

*CHD** Coronary heart disease; *DM*** Diabetes mellitus; *HPLC* High pressure liquid chromatography; *ELISA* Enzyme-linked immunosorbent assay
*** Healthy subjects living in urban slum, **** Healthy subjects living in an adjacent non-slum area

nomenon, being similar in all ethnic groups [19]. In the UK, however, a higher plasma Hcy level in Bangladeshis was put forth as an explanation for higher rates of CHD [35]. Since recent meta-analysis and reviews show a close relationship of hyperhomocysteinemia with CHD in other ethnic groups [38, 39], the lack of association in Asian Indians needs further scrutiny. Clearly, to establish a cause-effect relationship, more investigations especially cohort studies with long follow-up are required.

Another important observation of the current study is low intakes of vitamin B12 in 80.6% of subjects (71.4% males and 89.2% females) (Fig. 1). As we have, Refsum et al. also observed a high prevalence of cobalamine deficiency (47%), low holotranscobalamin (73%), and elevated serum methylmalonic acid (73%) in Western India [24]. In studies involving multi-ethnic populations, including Asian Indians, vitamin B12 appeared to be the main determinant of the difference in serum levels of Hcy. Chambers et al. recorded significantly lower levels of vitamin B12 in Asian Indians as

compared to the Caucasian population [18]. Asian Indians had lower mean levels of vitamin B12 as compared to Malays ($p < 0.01$) in Singapore [19]. Similar to the data on the levels of vitamin B12 in the current study, daily dietary intake of folic acid was below the RDA in 94.4 % of subjects (88.6 % males and all females) (Fig. 1). Low plasma folate was recorded in 41.9 % of males and 36.6 % of females in Asian Indians in Singapore, higher than that recorded in Chinese, and lower than those in Malays, though mean plasma folate levels were similar in all three ethnic groups [19]. Interestingly, serum folate levels were relatively high in the study of Refsum et al., and only 5 % of subjects were folate deficient [24]. A possible explanation for the differences in folic acid intake between the two groups in the present study could be that group I consumed more whole grain cereals while subjects in group II consumed more refined cereals and fast foods, relatively poor sources of this nutrient. Surprisingly, a high proportion of the subjects belonging to urban slums were non-vegetarians. This may be partially responsible for higher levels of vitamin B12 and other nutrients in group I as compared to group II, though the differences were statistically insignificant. The significant correlation of hyperhomocysteinemia with vegetarian status in the present study is well supported by other studies, where high levels of Hcy were observed with low B12 [40–42]. Additionally, vegetarians consistently showed poorer intakes of vitamin B12 and folic acid in both groups. In Refsum's study, however, low serum cobalamin levels were only partly explained by the vegetarian status of the population. In this study, cobalamin deficiency was common even in subjects having adequate eggs, poultry and mutton intake [24].

However, lack of correlation of lower intake of vitamin B12 and Hcy was an anomalous finding. It could be due to relatively small sample size, which would have an influence on the analysis of data in sub-groups, and is a limitation of the study. Further studies involving a larger sample size are needed to draw firm conclusions. Moreover, calculation of vitamin intakes by the nutrient recall method may be subject to inaccuracies and bias, though it was performed with maximum care and precision. Of note, while we have measured daily intake of nutrients, other studies record serum levels, and this may account for some of the observed discrepancies. Variations in the serum levels of the vitamin can be caused by a number of factors, resulting in further discrepancies in the calculated data of level of intake and serum levels. First, vitamin B12 may be consumed more than that determined by analysis of food proforma. For example, an additional small quantity of vitamin B12 in the vegetarian subjects may be provided by bacterial contamination of food items [26], a common occurrence in urban slums, and even in the hostel setting where most of the subjects of group 2 were residing. Second, a substantial amount of the vitamins may be lost when food is allowed to be cooked slowly over a prolonged period, a common practice in Asian households [43]. Finally, repeated bacterial, protozoal, and other gastrointestinal infections can cause a transient or prolonged state of poor nutrition and malabsorption [44–47]. This is often observed in the urban slums where sanitation is extremely unsatisfactory.

Among other possible causes of hyperhomocysteinemia, genetic mutations of the genes involved in the metabolism of Hcy, methylenetetrahydrofolate reductase (MTHFR) and cystathionine B-synthase (CBS) appear to be unlikely according to the available literature [48], which, however, needs further study.

Approximately 30–50 % of the population of major Indian cities now consists of urban slums [49]. The problem is serious and increasing because of escalating rural-urban migration. The high prevalence of coronary risk factors of urban slum dwellers in the same urban slum population has been recently recorded: hypercholesterolemia in 26.8 % and 27.5 %, hypertriglyceridemia in 16.8 % and 12.3 %, and high low-density lipoprotein cholesterol in 26 % and 25.4 % males and females respectively, in addition to high prevalence of abdominal obesity and glucose intolerance [22]. This population also consumes a pro-atherogenic diet: high in saturated fat, low in fiber and anti-oxidants and with a high prevalence of smoking [23]. Equally perturbing are hyperhomocysteinemia and deficient vitamin intakes in the apparently healthy subjects in non-slum area, particularly since this subset of the population is apparently health conscious and should consume balanced nutrition.

Though presently there is insufficient evidence in Asian Indians, the possibility exists that hyperhomocysteinemia may cause accelerated atherosclerosis, adding further to the high coronary risk. In addition, it may synergistically interact with the risk factors, e. g., lipoprotein (a) [Lp (a)]. This is particularly important since high Lp (a) levels in Asian Indians have been demonstrated [50], and co-existence of elevated serum levels of Lp (a) and Hcy have been predicted to increase the risk of CHD by nine fold [51].

The data of uniformly high levels of serum Hcy in urban dwellers, irrespective of the socio-economic class, raises significant concern, suggesting urgent employment of low-cost strategies for prevention of atherosclerosis and its complications in this semi-literate and illiterate population. First and foremost is to spread awareness about balanced nutrition and increased intake of fruits and vegetables. In addition, fortification of food materials with folate (e. g., grains [52, 53] and flour [54]), and its provision to people of low socio-economic strata at a subsidized cost should be considered in the National health policy. Cobalamin supplementation could also be considered for the CHD prevention in the population subgroups [43, 53], particularly in the devel-

oping countries where its deficiency is widespread [55]. These simple, inexpensive and effective interventions hold promise for reducing CHD prevalence and for simultaneous prevention of several vitamin deficiency-related disorders [17]. Micronutrients supplementation program and policy in India does not include such an option yet [56].

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