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Prevalence of vitamin deficiencies in an apparently healthy urban adult population: Assessed by subclinical status and dietary intakes

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ABSTRACT

Objectives: Studies in children and pregnant women consistently showed pandemic proportions of micronutrient deficiencies in the Indian subcontinent. However, vitamin deficiencies in apparently healthy adults are seldom recognized, hence the aim of this exploratory study was to assess their subclinical vitamin status and dietary intakes.
Methods: In all, 270 apparently healthy urban adults 30 to 70 y of age, from Hyderabad city, India participated in this study. Blood levels of vitamins (A, B_1, B_2, B_6, total and active B_{12}, D, and folate) and homocysteine were assessed. Anthropometric parameters were measured; dietary intake was obtained by food frequency questionnaire, and probability of adequacy (PA) was calculated by the estimated average requirement.
Results: Among the study population, the overall prevalence of deficiency of vitamin B_2 was strikingly high (50%) followed by the vitamins B_6 (46%), active B_{12} (46%), total B_{12} (37%), folate (32%), D (29%), B_1 (11%), and A (6%). Hyperhomocysteinemia (HHcys) was widely prevalent (52%) in the study participants. In case of dietary intakes, PA was lowest for vitamin B_{12} (4%) and folate (9%) followed by vitamins A (22%), B_2 (33%), B_6 (30%), and B_1 (59%). The mean PA of these vitamins was noticeably low (28%). The unadjusted logistic regression analysis found men and those with a deficiency of folate and total and active B_{12} to be at higher risk for HHcys. In the adjusted model, the risk for active B_{12} deficiency almost doubled.
Conclusion: The study demonstrated a high prevalence of multiple subclinical vitamin deficiencies, dietary inadequacies, and HHcys, which are possible risk factors for disease burden among apparently healthy adults.

Introduction

Vitamins are micronutrients essential for normal cellular and molecular functions, growth, and maintenance of body tissues [1]. Micronutrient deficiencies, referred to as *hidden hunger,* comprise vitamin and mineral deficiencies, which are propagated mostly through dietary inadequacies and are not apparent, but ubiquitous and affecting more than 2 billion people globally, with one-third of them residing in India [2].

Vitamin deficiencies continue to be the most critical risk factors for disease burden, especially in developing nations [3]. Despite the vital physiologic significance of all the vitamins, major human epidemiologic and intervention studies globally have focused on a select group consisting of folate, vitamin B_{12} and vitamin A owing to their widespread prevalence and distinct roles in health and disease [4]. Deficiencies of other vitamins have hitherto received relatively less attention, probably owing to the unavailability of simple analytical assays and a lack of globally established cutoffs. However, it is noteworthy that deficiency of other vitamins, either independently or in combination, results in deleterious consequences. Studies show that thiamine deficiency is implicated in dementia, Alzheimer's disease, cancer, and metabolic diseases [5]. Similarly, severe riboflavin deficiency is associated with neurodegeneration, peripheral neuropathy, cancer, and cardiovascular diseases [6].

GBR, M Sivaprasad, and TS designed the research. M Sivaprasad, TS, PYR, and M Seshacharyulu conducted the research. GBR provided essential reagents and materials. M Sivaprasad, TS, GM, and BNK analyzed the data or performed the statistical analysis. M Sivaprasad, TS, and GBR wrote the paper. GBR takes primary responsibility for the final content. All authors read and approved the final manuscript. GBR acknowledges financial assistance from the Department of Science and Technology, Government of India (grant no. SB/SO/HS-192/2013) and the Department of Biotechnology, Government of India (grant no. BT/PR10658/PFN/20/806/2013). M Sivaprasad and TS acknowledge a research fellowship from Indian Council of Medical Research, Government of India.
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Vitamin B_6 deficiency is associated with cognitive failure, seizures, cancer, migraine, chronic pain, cardiovascular events, immune deficiency, and depression [7]. Hence, it would be prudent to extend our focus to these vitamins as well.

Clinical signs and overt pathology of vitamin deficiencies differ among the population according to age and sex and are more likely to precipitate into clinical deficiencies in vulnerable groups such as children and pregnant and lactating women. However, clinical signs of vitamin deficiencies represent only the "tip of the iceberg" of the magnitude of the problem, whereas subclinical deficiencies of vitamins concealed in the apparently healthy adult population are widespread and are equally devastating [8,9]. Our recent studies indicated the alarmingly high prevalence of deficiency of vitamin B_{12} (41%) and vitamin D (45%) among apparently healthy adults [10,11]. A subsequent study further reinforced these findings with a similar prevalence of vitamin B_{12} deficiency, along with suboptimal dietary intakes [12]. These studies emphasize that apparently healthy adults who rarely get their vitamin levels screened may be silent victims of possible subclinical vitamin deficiencies. Because this population constitutes the primary workforce, these deficiencies can have direct implications on the economic productivity of the nation [13]. Considering the contribution of vitamin deficiencies to many age-related non-communicable diseases (NCDs), assessing the magnitude of vitamin deficiencies among healthy adults is vital, but, to our knowledge, very few studies are available [14,15] in this context. The accomplishment of this end presupposes knowledge of the prevalence of vitamin deficiencies and associated factors for the development of appropriate intervention strategies for their control and treatment. Therefore, an exploratory study was conducted among apparently healthy adults to assess the subclinical status of vitamins (A, D, B_1, B_2, B_6, B_{12}, and folate) along with their dietary intakes.

Participants and methods

Study design, selection, and sample size

A community-based, cross-sectional exploratory study was conducted from July 2016 to June 2017 predominantly in an urban setup. The volunteers enrolled in the study were apparently healthy adults 30 to 70 y of age from Hyderabad, Telangana, a southern state in India. The recruitment process was carried out by organizing health camps at randomly selected locations of Hyderabad to capture the entire population of the city. Children and pregnant and lactating women were not included. A random number of volunteers (N = 450) without any signs of clinical conditions or the need for medical treatment, were enrolled and informed about the study. Among them, 63 declined to participate in the diet survey; hence 387 individuals willing to participate were screened for eligibility by assessing their health status with a questionnaire. In all, 82 individuals who reported chronic alcohol abuse, hyperglycemia, renal problems, severe metabolic complications or surgery of the gastrointestinal tract, multivitamin supplement usage for the past 6 mo, or an acute illness at the time of enrollment were excluded, resulting in 305 eligible participants.

Twenty-eight individuals who did not turn up after enrollment, and seven who did not provide complete information, were excluded. Individuals with a history of controlled hypertension were retained, and relevant information was recorded. Finally, 270 participants (147 men and 123 women) who fulfilled the criteria consented and were sampled as shown in Figure 1.

The study followed the guidelines laid down in the Declaration of Helsinki, and its later amendments and all protocols involving human subjects were approved by the Institutional Ethics Committee of the National Institute of Nutrition, Hyderabad, India. After obtaining the written consent from all the participants, 6 mL of fasting venous blood was collected in heparin tubes, and 5 mL of the first-morning void urine sample was collected in sterile containers. Blood and plasma (separated by centrifugation of blood at 3500g/10 min) were aliquoted appropriately. Analysis of all the clinical parameters was carried out on the same day, and remaining sample aliquots were stored at −80°C for the estimation of vitamins and homocysteine. Demographic and socioeconomic data of the participants was collected using a validated questionnaire.

Anthropometric measurements

The body weight (to the nearest 0.1 kg) and height (to the nearest 0.1 cm) were recorded using the SECA weighing scale (Deutschland, Medical Scales, and Measuring System) and anthropometric rod, respectively. Body mass index (BMI) was calculated using Quetelet's index (kg/m^2). Waist circumference (WC) was measured at a point midway between the lower rib margin and the iliac crest using a fiber-reinforced nonelastic tape. Blood pressure (BP) was monitored by a digital blood pressure apparatus (HEM-7111 model; Omron Healthcare Co. Ltd) at 5-min intervals in the sitting position in triplicates, and the average of three such readings was taken [16].

Biochemical estimations

Fasting blood glucose (FBG) was estimated in whole blood using a glucometer (Accu-Chek Active Roche Diagnostics GmbH, Mannheim, Germany) [17]. Glycosylated hemoglobin (HbA1c) was estimated in whole blood by Afinion AS100 Analyser (Axis-Shield, Norway) based on the principle of fully automated boronate affinity assay [18] and Hb by the cyanmethemoglobin method using a spectrophotometer (Shimadzu UV 2600). Lipid profile (total cholesterol [TC], triacylglycerols [TG], high-density lipoprotein cholesterol) was analyzed in plasma using commercially available kits from BioSystems (Barcelona, Spain). Low-density lipoprotein cholesterol concentrations were calculated using the Friedewald formula. Renal function was evaluated by measuring plasma creatinine based on the modified kinetic Jaffe method using the kit from BioSystems. Moderately elevated albuminuria, an independent risk factor for cardiovascular disease [19] and renal dysfunction [20] in the general population, was screened by measuring the urinary albumin-to-creatinine ratio (UACR; expressed as mg/g creatinine) [21]. Urinary albumin was quantified using a solid-phase immunochemical assay and urinary creatinine by an enzymatic colorimetric test in a fully automated Afinion AS100 Analyser (Axis-Shield, Norway) [22]. The cutoff values used for the anthropometric and biochemical parameters are shown in Table 1.

Estimation of vitamins and total homocysteine

Vitamin A was measured in plasma by a reverse phase high-performance liquid chromatography (HPLC) column coupled with ultraviolet detection according to Bieri et al. [23]. The active biological forms of vitamins B_1, B_2, and B_6: thiamine pyrophosphate (TPP), flavin adenine dinucleotide (FAD), and pyridoxal-5′-phosphate (PLP), respectively, were measured in whole blood (TPP and FAD) or in plasma (PLP) using commercially available reverse-phase HPLC kit with fluorescence detection (Recipe Chemicals + Instruments GmbH, Germany) [10]. Enzyme-linked immunosorbent assay was used to quantify 25-hydroxyvitamin D as per the manufacturer's instructions [24]. Simultaneous analysis of plasma levels of total B_{12} and folate were carried out using a commercially available solid phase radioimmunoassay kit (MP Biomedicals, Diagnostic Division, New York, NY, USA) [12]. A gamma counter equipped with dual-channel for determining the radioactivity of ^{57}Co and ^{125}I simultaneously was used (PerkinElmer, 3 wizard 1480, Waltham, MA, USA). Vitamin B_{12} bound to transcobalamin II is considered biologically active [25] (also called active B_{12}) and was estimated in plasma by enzyme immunoassay (Axis-Shield Diagnostics, Dundee, Scotland, UK) as per the manufacturer's instructions. Plasma total homocysteine (tHcys) was determined by employing a special reverse phase HPLC column with fluorescence detection (Recipe Chemicals Instruments GmbH, Germany) [12]. The cutoff values used for vitamins and homocysteine are shown in Table 2.

Dietary assessment

Individual dietary intake was assessed in a subset of the samples (n = 111, 55 men, 56 women) using systematic random sampling procedure. A validated raw food-based food frequency questionnaire (RFFQ) of 1-y duration was used for dietary assessment. The RFFQ comprised a list of 141 raw food items along with the corresponding food frequency responses and quantity of raw food consumed [26]. A standardized set of 12 cups and 2 spoons were used as visual tools for the administration of the diet survey and for assessing the portion sizes. The raw ingredients used for the food preparation were weighed using a portable electronic digital diet scale (Seca Culina 852) with 1-g accuracy [27].

The nutritive value of raw foods was calculated using the new Indian Food Composition Tables [28], and for the ones that were missing, the US Department of Agriculture food and nutrient database was used [29]. The nutritive values of these two databases were compared after correction for moisture values, and the variations were found to be comparable. Food and nutrient intake from the RFFQ were calculated based on individual consumption unit (CU) and were computed using the in-house software. An adult man doing sedentary work was considered as one (unit). Furthermore, arbitrary calorie coefficient values were assigned to individuals of different age, sex, and activity groups [30]. Finally, the food intake of one participant was calculated by converting the different frequencies of food consumption to per day intake [27].

$$\text{Food intake (in g or ml)} = [\text{Total Quantity of Food (in g or ml)}/\text{Total CU}] * \text{Individual CU} * \text{Frequency of consumption}$$

Assessment of dietary inadequacy of nutrients at the population level was carried out using the probability approach method, which is based on the comparison of the univariate distribution of nutrient requirement and the usual nutrient intake.

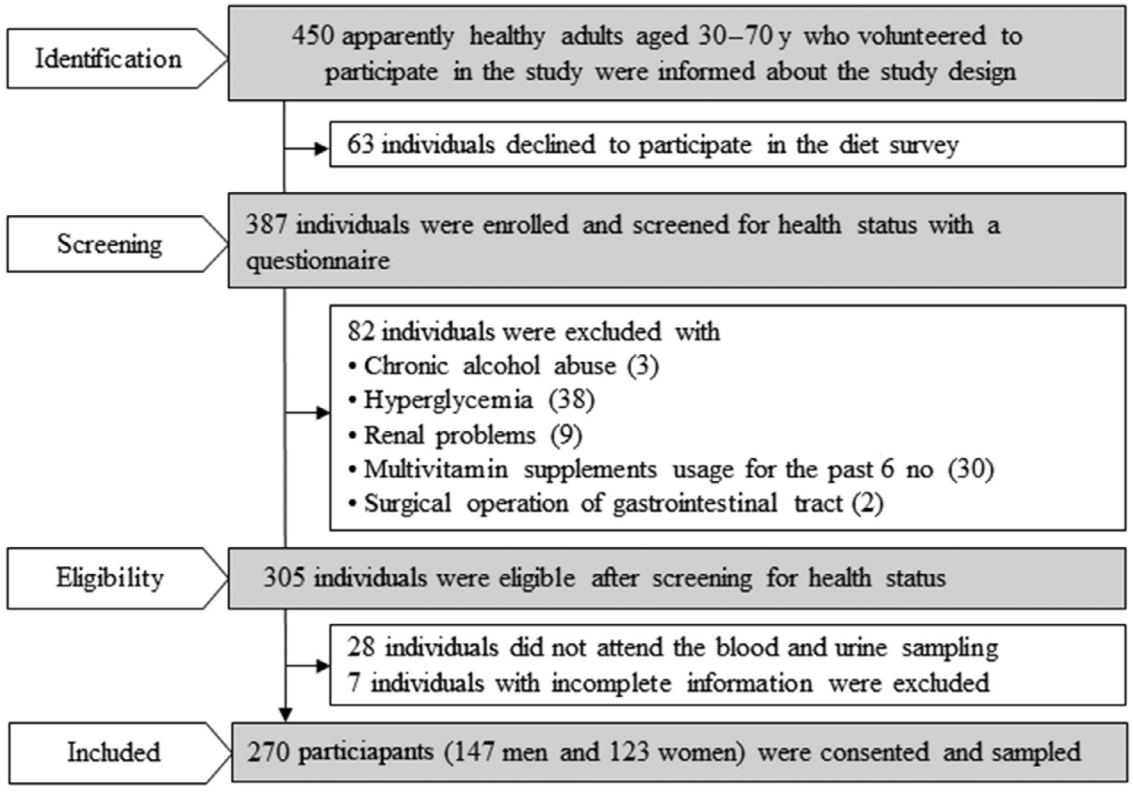

Fig. 1. Flowchart showing the selection and recruitment of study participants.

A continuous risk probability function is applied to the individual's intake, and the individual probabilities are subsequently averaged across the population. The individual intake data is plotted from the study population against the risk curve constructed using the information on the requirement distribution (Estimated Average Requirement [EAR] and its SD). Furthermore, the proportion of the population with an inadequate intake is estimated by comparing the risk curve to the distribution of usual intakes for the population. The mean of the individual probabilities was used to estimate the prevalence of inadequacy of a particular nutrient [31].

Based on this distribution, the "cdfnorm" function in SPSS was used to compute the probability of adequacy (PA). The recommended EAR according to sex and age group were followed as set by the Institute of Medicine (IOM; National Academies, Food and Nutrition Board; Table 2). The PA of six vitamins (vitamins A, B_1, B_2, B_6, folate, and B_{12}) was calculated. The mean probability of adequacy (MPA) refers to the average of PAs for the six vitamins.

Table 1
Cutoff values used for the anthropometric and clinical parameters

Parameter	Cutoff values	Reference
BMI, kg/m^2	Chronic energy deficiency (<18.5) Normal (18.5–22.99) Over Weight (23–27.49) Obese (≥27.5)	[53]
WC, cm	Normal (M<90, W<80)	[54]
SBP, mm Hg	Normal (<140)	[55]
DBP, mm Hg	Normal (<90)	[55]
FBG, mg/dL	Normal (<110) IGT (110–126) Diabetes (≥126)	[56]
TC, mg/dL	Normal (<200)	[57]
HDL-C, mg/dL	Normal (M≥40, W≥50)	[57]
LDL-C, mg/dL	Normal (<130)	[57]
TG, mg/dL	Normal (<150)	[57]
Hb, g/dL	Normal (M>13, W >12)	[58]
HbA1c, %	Normal (<6.4)	[59]
Plasma creatinine, mg/dL	Normal: M (0.7–1.2), W (0.5–0.9)	[60]
UACR, mg/g creatinine	Normal to mildly increased (<30) Moderately increased (30–300) Severely increased (≥300)	[61]

BMI, body mass index; DBP, diastolic blood pressure; FBG, fasting blood glucose; Hb, hemoglobin; HbA1c, glycosylated hemoglobin; HDL-C, high-density lipoprotein cholesterol; IGT, impaired glucose tolerance; LDL-C, low-density lipoprotein cholesterol; M, men; SBP, systolic blood pressure; TC, total cholesterol; TG, triacylglycerols; UACR, urinary albumin to creatinine ratio; W, women; WC, waist circumference;.

Statistical analyses

Data processing and statistical analyses were performed with SPSS version 19 (SPSS Inc, Chicago, IL, USA) and R software package. Because most of the data were skewed, the population characteristics were reported using medians, 25th (P_{25}) and 75th (P_{75}) percentiles, and comparisons for the same were carried out by the Mann–Whitney U test. The χ^2 test was used for comparison of the prevalence of vitamin deficiencies. Student's t test was used to compare the PA and MPA between the sexes. A Spearman's rank correlation analysis was carried out to evaluate the correlation coefficients among the clinical and biochemical parameters on a scale of −1 to +1. Logistic regression analysis was applied to examine the association of age, sex, and vitamin status with hyperhomocysteinemia (HHcys). The level of significance was considered at $P < 0.05$.

Results

The median values and 25th and 75th percentiles of anthropometric, clinical, and biochemical characteristics of the participants by sex are shown in Table 3. The study population was 54% (n = 147) men and 46% women (n = 123). The median values of vitamins A, B_1, and tHcys were considerably higher in men than in women; whereas other vitamins were not significantly different.

About 4.5% of the participants had low body mass index and suffered from chronic energy deficiency; whereas 45% were overweight and 28% were obese. Around 68% of the participants

Table 2
Cutoff values of vitamins and homocysteine and reference values of dietary intakes (EARs) of vitamins

	Blood and plasma levels			Dietary Intake
Parameter	Cutoff to define deficiency	Sample	Reference	EAR values, CV% [62]
Vitamin A	≤0.7 μmol/L	Plasma	[63]	M: 625 μg/d, 20 W: 500 μg/d, 20
Vitamin D	<27.5 nmol/L	Plasma	[64]	NA
Vitamin B_1* (TPP)	<66 nmol/L	Whole blood	RECIPE† Chemicals + Instruments GmbH. (Cat. No. 27000)	M: 1 mg/d, 10 W: 0.9 mg/d, 10
Vitamin B_2* (FAD)	<228 nmol/L	Whole blood	RECIPE† Chemicals + Instruments GmbH. (Cat. No. 27000)	M: 1.1 mg/d, 10 W: 0.9 mg/d, 10
Vitamin B_6* (PLP)	Mild: <20 nmol/L Severe: <10 nmol/L	Plasma	[64]	M, 19–50 y: 1.1 mg/d, 10 ≥51 y: 1.4 mg/d, 10 W, 19 –50 y: 1.1 mg/d, 10 ≥51 y: 1.3 mg/d, 10
Folate	<10 nmol/L	Plasma	[64]	320 μg/d, 10
Total B_{12}	<150 pmol/L	Plasma	[64]	2 μg/d, 10
Active B_{12}	<35 pmol/L	Plasma	[65]	NA
tHcys	>15 μmol/L	Plasma	[52]	NA

CV, coefficient of variation; EAR, estimated average intake; FAD, flavin adenine dinucleotide; M, men; NA, not available; PLP, pyridoxal-5′-phosphate; tHcys, total homocysteine; TPP, thiamine pyrophosphate; W, women
*For vitamins B_1, B_2, and B_6 the corresponding active forms of TPP, FAD, and PLP were considered for determining the cutoff values.
†In this study, TPP and FAD were estimated in whole blood, but because there are no agreed cutoffs for the same, we used the lower limit of the normal ranges mentioned by the manufacturer as cutoff values.

(62% of men and 73% of women) had abdominal obesity as assessed by waist circumference. The overall prevalence of anemia was 20% (16% men and 26% women) and hypertension was 33%. The prevalence of hypercholesterolemia was 22%, hypertriacylglycerolmia was 21%, high low-density lipoprotein levels were 25%, and low high-density lipoprotein levels were 67%. Moderately elevated albuminuria was observed in 10% of the participants.

The overall prevalence of deficiency of vitamins A, D, B_1, B_2, B_6, and folate were 6%, 29%, 11%, 50%, 46%, and 32%, respectively (Fig. 2). The prevalence of deficiency of vitamin B_6 was 30% in the mild form and 16% in the severe form. The prevalence of deficiency

Table 3
Anthropometric, clinical, and biochemical profile of the study participants by sex

Parameter	Men (n = 147) Median (P_{25}–P_{75})	Women (n = 123) Median (P_{25}–P_{75})	Pooled (N = 270) Median (P_{25}–P_{75})	*P*-value
Age, y	60 [a] (48–65)	56 [a] (45–62)	59 (45–64)	0.347
Height, m	1.60 [a] (1.6–1.7)	1.54 [b] (1.50–1.57)	1.6 (1.53–1.67)	<0.001*
Weight, kg	68.9 [a] (60.8–70.8)	61.2 [b] (51.9–70.2)	65 (56.7–73.1)	<0.001*
BMI, kg/m^2	24.6 [a] (22.5–27.1)	25.7 [a] (22.7–29.2)	24.9 (22.7–27.8)	0.258
WC, cm	94 [a] (86.4–99.1)	86.9 [b] (79.8–94)	89.9 (83.8–97.8)	0.004*
SBP, mm Hg	132 [a] (120–153)	130 [a] (118–147)	132 (118.5–150.5)	0.897
DBP, mm Hg	80 [a] (71–91)	79 [a] (74–90)	80 (73–90.5)	0.980
FBG, mg/dL	96 [a] (89–108)	98 [a] (92–105)	98 (91–106)	0.955
TC, mg/dL	163.2 [a] (142–194)	176.1 [b] (149.1–196.8)	169.7 (144.6–195.8)	0.030*
HDL-C, mg/dL	35.8 [a] (29.6–42.5)	41.0 [b] (34.4–51.4)	38.2 (31.3–45.8)	0.002*
LDL-C, mg/dL	101 [a] (85–125.1)	112.8 [a] (86.6–132.4)	104.1 (85.5–130)	0.074
TG, mg/dL	113.4 [a] (71.4–155.6)	83.9 [b] (66.4–111.8)	92.1 (68–140)	<0.001*
Hb, g/dL	14.6 [a] (13.4–15.7)	12.4 [b] (11.5–13.5)	13.5 (12–15)	<0.001*
HbA1c, %	5.7 [a] (5.5–5.9)	5.8 [a] (5.5–5.9)	5.8 (5.5–5.9)	0.246
Creatinine, mg/dL	1.1 [a] (1–1.15)	0.9 [b] (0.8–0.9)	1 (0.8–1.1)	<0.001*
UACR, mg/g creatinine	9.8 [a] (6–17.7)	13.7 [a] (6.8–25.1)	10.9 (6.1–22.5)	0.149
Vitamin A, μmol/L	1.4 [a] (1.1–1.7)	1.1 [b] (0.9–1.4)	1.3 (1–1.6)	0.002*
Vitamin D, nmol/L	35.4 [a] (26.7–49.3)	35.8 [a] (22.6–58.6)	35.6 (24.7–54.1)	0.903
Vitamin B_1, nmol/L	132.8 [a] (94.2–158.8)	112.1 [b] (93.2–134.0)	119.6 (93.5–147.1)	0.034†
Vitamin B_2, nmol/L	217.5 [a] (182–287.7)	241.9 [a] (191–300)	222 (184.1–292)	0.220
Vitamin B_6, nmol/L	21.9 [a] (13–31)	21.5 [a] (12.7–32.8)	21.7 (13–32)	0.771
Folate, nmol/L	12.3 [a] (7.9–20.4)	13.1 [a] (8.9–21.2)	12.7 (8.4–20.7)	0.762
Total B_{12}, pmol/L	169.7 [a] (118.1–258.3)	204.4 [a] (135.0–310.2)	184.5 (125.4–280.4)	0.051
Active B_{12}, pmol/L	41 [a] (20.1–59.3)	38.8 [a] (28.3–70.4)	40.0 (22.6–68.3)	0.210
tHcys, μmol/L	20.4 [a] (12.9–33.6)	12.3 [b] (9–21.3)	15.6 (10.2–29.3)	<0.001*

BMI, body mass index; DBP, diastolic blood pressure; FBG, fasting blood glucose; Hb, hemoglobin; HbA1c, glycosylated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; P_{25}, 25th percentile; P_{75}, 75th percentile; SBP, systolic blood pressure; TC, total cholesterol; TG, triacylglycerols; tHcys, total homocysteine; UACR, urinary albumin to creatinine ratio; WC, waist circumference
Values represent medians, 25th, and 75th percentiles. Significant differences ($P < 0.05$; $P < 0.01$) of median values between the sexes are indicated by different superscript letters (a, b)
*Significantly different at $P < 0.01$.
†Significantly different at $P < 0.05$.

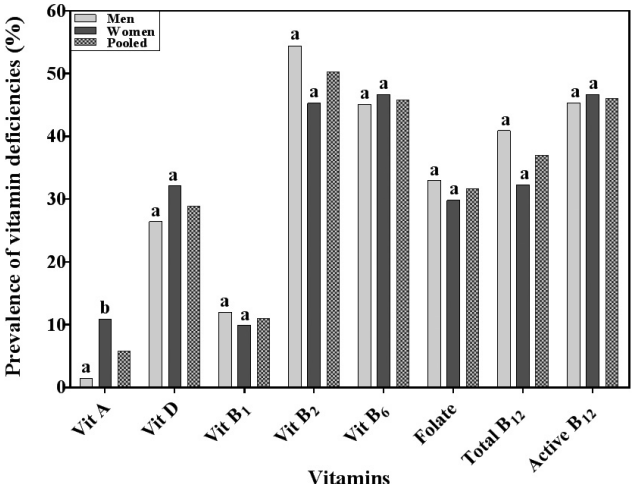

Fig. 2. Prevalence of vitamin deficiencies in apparently healthy adults. Pooled data represents the total number of samples (N = 270). Data represent % deficiency, and significant differences ($P < 0.05$) of mean values between the sexes are indicated by letters (a, b) above the bars.

of total B_{12} was 37%; whereas that of active B_{12} was 46%. Interestingly, HHcys was observed in 52% of the participants and was significantly higher in men (66.7%) than in women (36.4%; Fig. 3).

The median dietary intakes are shown in Table 4. Except for vitamin B_{12}, the median dietary intakes of all other vitamins were significantly different between the sexes and were higher in men than in women. The PA of vitamins between the sexes is shown in Figure 4. Among all the B vitamins tested, the PA was lowest for vitamin B_{12} (4%) and folate (9%). The adequacy of vitamin B_1 was significantly higher in men than in women. Noticeably, the MPA was only 28% and was significantly higher in men (34%) than in women (22%). The significant correlations of blood and plasma levels of vitamins with clinical parameters were as follows:

- Vitamin A was positively correlated with Hb.
- Vitamin D was associated negatively with FBG.
- Folate had a positive association with total and active B_{12}.
- Total B_{12} was positively correlated with active B_{12}.
- tHcys was negatively correlated with folate and vitamin B_{12}.

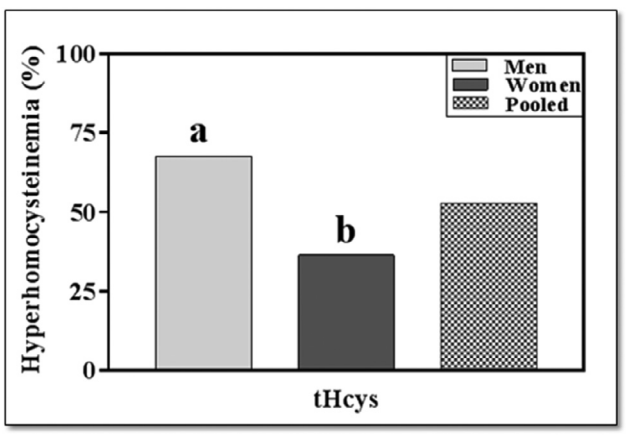

Fig. 3. Prevalence of hyperhomocysteinemia in the study population. Pooled data represents the total number of samples (N = 270). Data represent % deficiency, and significant differences ($P < 0.05$) of mean values between the sexes are indicated by letters (a, b) above the bars. tHcys, total homocysteine.

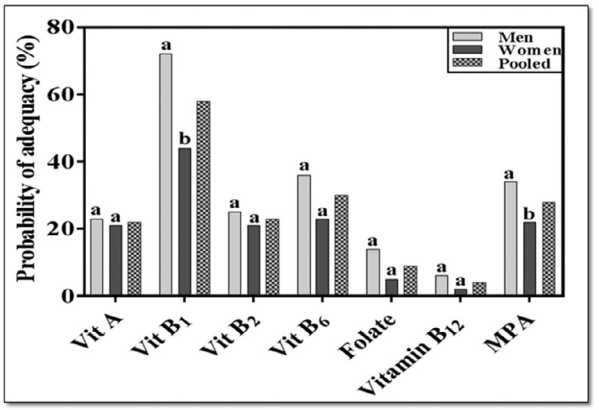

Fig. 4. Probability of adequacy of vitamins in the study participants. Pooled data represents the total number of samples (N = 111). The MPA is defined as the mean of the probability of adequacy across the six vitamins. Data represent % adequacy, and significant differences ($P < 0.05$) of mean values between the sexes are indicated by letters (a, b) above the bars. MPA, mean probability of adequacy.

The risk for HHcys and its association with different determinants such as sex and vitamin status was compared by calculating the odds ratios (OR) with logistic regression analysis (Table 5). In the unadjusted group, the risk for HHcys was higher in men (OR, 3.5; 95% confidence interval [CI], 1.7–7) and in individuals with deficiencies in folate (OR, 2.9; 95% CI, 1.3–6.5), total B_{12} (OR, 2.4; 95% CI, 1–5.2), and active B_{12} (OR, 2.8; 95% CI, 1.1–7). When adjusted for age and sex, the risk for active B_{12} deficiency almost doubled in the adjusted group (OR, 5.8; 95% CI, 1.7–19.7) compared with the unadjusted one.

Discussion

Micronutrient malnutrition comprising vitamin deficiencies leads to exorbitantly high mortality and morbidity [2]. It has been an unrelenting problem throughout the developing world, especially in southern Asia and sub-Saharan Africa [3]. The pervasiveness of vitamin deficiency diseases plagues the Indian subcontinent and studies consistently demonstrated a high prevalence of vitamin D, folate, and B_{12} deficiencies. However, other vitamins such as B_1, B_2, and B_6 have received less attention, possibly owing to the unavailability of widely used assay procedures.

Clinical signs of vitamin deficiency represent a small portion of the affected population that otherwise suffers from subclinical vitamin deficiencies that are widespread and pose a potential threat of disease burden. Apparently healthy individuals with subclinical vitamin deficiencies might appear normal, hence, they are likely to receive less attention but often experience increased susceptibility to infections, oxidative stress, and degenerative diseases [32]. The present study provided insight into the lurking dangers of multiple subclinical vitamin deficiencies concealed in the apparently healthy population. The study considered the possible associations between the anthropometric and clinical parameters with vitamin status and tHcys levels. Because micronutrient deficiencies mainly stem from inadequate diet, nutrient adequacy of the study population was assessed by the probability approach method, which enables one to evaluate dietary intake data with higher accuracy. The clinical and comorbid status and dietary inadequacies observed in this study are comparable with other reported surveys in the Indian context.

Earlier, the evaluation of thiamine and riboflavin deficiency was broadly determined by indirect measurement of the erythrocyte thiamine transketolase activity coefficient and erythrocyte

Table 4
Medians, P_{25}, and P_{75} of dietary intake of vitamins by sex

Parameter	Sex			
	Men (n = 55) Median (P_{25}–P_{75})	Women (n = 56) Median (P_{25}–P_{75})	Pooled (N = 111) Median (P_{25}–P_{75})	*P*-value
Vitamin A, μg/d	405.6[a] (282.7–565)	293.6 [b] (213.2–465.6)	336 (251.5–499.4)	0.006*
Vitamin B_1, mg/d	1.2 [a] (1–1.3)	0.8 [b] (0.6–1.1)	1 (0.8–1.2)	<0.001*
Vitamin B_2, mg/d	0.9 [a] (0.7–1.1)	0.7 [b] (0.5–0.9)	0.8 (0.6–1)	<0.001*
Vitamin B_6, mg/d	1.2 [a] (1–1.4)	1.0 [b] (0.8–1.15)	1.1 (0.9–1.3)	<0.001*
Folate, μg/d	219.1 [a] (185.4–270.4)	173 [b] (143.5–230)	198.4 (156.9–258.1)	0.006*
Vitamin B_{12}, μg/d	0.4 [a] (0.3–0.9)	0.5 [a] (0.3–0.9)	0.5 (0.3–0.9)	0.928

PA, probability of adequacy; P_{25}, 25th percentile; P_{75}, 75th percentile
Values represent medians, 25th and 75th percentiles and percentages. The mean probability of adequacy is defined as the mean of the PA across the six vitamins. Significant differences of median values and frequencies between the sexes ($P < 0.01$) are indicated by different superscript letters (a, b)
*Significantly different at $P < 0.01$.

glutathione reductase activity coefficient respectively. In this study, the HPLC method for the direct determination of thiamine (TPP) and riboflavin (FAD) in whole blood was used, which gives the actual status of these vitamins in addition to other advantages regarding higher precision, sensitivity, specificity, and robustness [33,34].

Vitamin B_2 deficiency is widespread in half of the study population, affecting both sexes (54.4% men and 45.3% women) along with a high dietary inadequacy of 77%. This rampant deficiency is not only quite alarming but is in contrast to the commonly highlighted vitamin deficiencies such as vitamins A, B_{12}, D, and folate. In a study carried out in the Indian rural population by National Nutrition Monitoring Bureau (NNMB), low median intakes of vitamin B_2 were reported against the Recommended Dietary Allowance (RDA) in all the states [35]. Further analysis of this data revealed the high dietary inadequacy of vitamin B_2 (71%) (NNMB unpublished observations). Because riboflavin is primarily present in the germ and barn, milling of rice and wheat might result in considerable losses of the vitamin [36]. Polished grains are now preferred over unpolished ones in both rural and urban areas and can potentially contribute to riboflavin deficiency. To the best of our knowledge, the prevalence of vitamin B_2 deficiency by the direct determination of FAD concentration in whole blood by the HPLC method along with its dietary intake in healthy Indian adults is reported for the first time.

The overall prevalence of deficiency of vitamin B_6 (measured as PLP) was remarkably high (45.8%), which could be attributed to its high dietary inadequacy (70%), which is commonly not reported in the Indian scenario. Pyridoxal phosphate, the active form of vitamin B_6 requires vitamin B_2 for its conversion from pyridoxine [32], and high deficiency of vitamin B_2 could have influenced its status. Prevalence of deficiency of total vitamin B_{12} was 37%, which is similar (35–40%) to our previous findings in the healthy adults [10,12]. Active B_{12} is an early marker [37] with a better representation of the actual vitamin B_{12} status [38–40], and interestingly it predicted a higher prevalence of deficiency in the present study. The overall prevalence of folate deficiency was 32%, which might have been influenced by the status of other B vitamins. Folate requires vitamins B_2 and B_{12} to maintain as tetrahydrofolate [32] (active reduced form), and their deficiency might have a role in lowered folate levels. A positive correlation between plasma folate and vitamin B_{12} (total and active) in the study participants reaffirms this interrelationship. A remarkably high dietary inadequacy of vitamin B_{12} (96%) and folate (91%) was observed in the study population. Analysis of NNMB data revealed similar results with a high prevalence of dietary inadequacy of vitamin B_{12} (99%) and

Table 5
Unadjusted and adjusted OR and 95% CIs of elevated tHcys concentrations: Results from logistic regression analyses

Variable	tHcys >15 μmol/L					
	Unadjusted			Adjusted*		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Sex						
Women	1			–	–	–
Men	3.5	(1.7–7.0)	<0.001†	–	–	–
Vitamin B_6 (nmol/L)						
≥20	1			1		
10 to 20	2.1	(0.8–4.9)	0.095	2.1	(0.8–5.2)	0.111
<10	2.8	(0.7–10.3)	0.127	3.7	(0.9–15.1)	0.068
Folate (nmol/L)						
>10	1			1		
≤10	2.9	(1.3–6.5)	0.008†	3.4	(1.4–8.1)	0.006†
Total B_{12} (pmol/L)						
>150	1			1		
≤150	2.4	(1–5.2)	0.032‡	3	(1.2–7.5)	0.015‡
Active B_{12} (pmol/l)						
>35	1			1		
≤35	2.8	(1.1–7)	0.029‡	5.8	(1.7–19.7)	0.005†

tHcys, total homocysteine
*Adjusted for age and sex.
†Significantly different at $P < 0.01$.
‡Significantly different at $P < 0.05$.

folate (93%) in the rural population (NNMB unpublished observations). Such high dietary inadequacies, coupled with subclinical vitamin deficiencies, can predispose the individuals to chronic diseases [12]. Despite having abundant sunshine, Indians are prone to vitamin D deficiency [41]. The overall prevalence of subclinical vitamin D deficiency in the present study was 29%. An inverse correlation was observed between FBG, and vitamin D levels of the study participants and similar findings were reported in a European study on older individuals [42], indicating the role of vitamin D in glucose metabolism. Among the B vitamins, vitamin B_1 had the least prevalence of deficiency (11%) with a dietary inadequacy of 41%. The prevalence of deficiency of vitamin A was very low in the study participants despite a dietary inadequacy of 78%. A high dietary inadequacy of vitamin A (84%) was also noticed in rural Indians (NNMB unpublished observations). An Indian study reported significantly low plasma retinol levels in women compared with men [43] and similar findings were noted in the present study. The positive association of vitamin A with Hb levels observed in the present study implies its cross-talk with iron metabolism [44]. Interestingly, studies also reported a beneficial effect of vitamin A supplementation along with iron on Hb and other iron status markers [45,46].

HHcys, implicated in cerebrovascular and cardiovascular diseases, osteoporosis-associated fractures, and dementia-type disorders [47], has been a frequent trait in all Indians, including those residing in other parts of the globe [48]. We found a significant percentage of the study participants with HHcys (52%), which has been a consistent observation by several research groups in healthy Asian Indians [49]. Significantly high levels of tHcys were observed in men compared with women. Vitamins B_{12} and folate are known to be integral players of the homocysteine metabolism [50], which is reflected by the inverse association of tHcys with plasma levels of folate and vitamin B_{12} (total and active). A high degree of HHcys might have resulted from high dietary inadequacies of vitamin B_{12} and folate.

HHcys also involves the deficiency of B vitamins [51] other than vitamin B_{12} and folate. Studies have shown the involvement of vitamin B_6 in remethylation and vitamin B_2 in transsulfuration of homocysteine [6]. Hence, we suspect that a high deficiency of B_2 and B_6 might have further contributed to HHcys in the study population. In unadjusted logistic regression analyses, elevated homocysteine was used as the outcome variable that found male sex and low concentrations of folate, total B_{12}, and active B_{12} to be significantly associated with HHcys and are in line with other findings [52]. Interestingly, the risk for active B_{12} deficiency almost doubled in the age and sex-adjusted model, which underlines its predictive power over total B_{12} and its inverse relationship with homocysteine.

Although there is a low prevalence of chronic energy deficiency, markedly high micronutrient malnutrition is observed as evidenced by remarkably low MPA of 28% in the study population. The Indian diet is calorie dense, consisting mainly of cereal/pulse-based foods accompanied by inadequate intakes of vegetables, fruits, and dairy products and thus fails to meet the vitamin requirements. Furthermore, the nation is in a state of nutritional transition where undernutrition runs parallel with overweight and obesity (particularly abdominal adiposity) as evidenced by the high prevalence of these two conditions in the study population. In the present study, although men had significantly higher intakes of the six vitamins than women, the same trend was not reflected in their blood levels. Discrepancies between the biochemical status and dietary intakes underline the involvement of non-dietary factors such as age, environment, genetics, bioavailability, malabsorption, nutritional disorders, and status of other nutrients.

Conclusion

Vitamin deficiencies are ubiquitous, yet remain clinically undetectable unless severe. However, even a mild form of vitamin deficiency can result in adverse consequences. The findings of the present study demonstrated the widespread prevalence of multiple subclinical vitamin deficiencies and inadequacy of vitamin intakes in an apparently healthy adult population, with women being particularly at higher risk. In addition, a high degree of HHcys was observed, which amplifies the chances of non-communicable diseases. Interestingly, a very high burden of B_2 and B_6 deficiencies was noticed in addition to the commonly reported vitamin deficiencies such as folate and vitamins B_{12}, D, and A. Active B_{12} emerged as a better predictor of vitamin B_{12} status than total B_{12}. It is evident from the study that subclinical vitamin deficiencies are insidious in apparently healthy adults; hence it would be prudent to screen them regularly. This study can provide firsthand information to researchers, medical professionals, and policymakers regarding the magnitude of the prevailing situation. Furthermore, it suggests the necessity for nationally representative data to direct the improvement of nutrition interventions and public health programs, such as multiple micronutrient fortifications, dietary diversification, and supplementation to achieve the sustainable developmental goals.

Strengths and limitations

A comprehensive evaluation of vitamins of public health importance along with their dietary intake was carried out. Direct estimation was used in place of enzyme activation assays for the analysis of vitamins B_1, B_2, and B_6. The probability approach method, which enables the evaluation of dietary intake data with higher accuracy, was used. Active B_{12}, which was a better predictor of vitamin B_{12} status, was analyzed. The study population might not be representative of the entire nation concerning geography, food habits, and other cultural variations, which warrants further studies with larger cohorts.

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