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ORIGINAL CONTRIBUTION

Micronutrient intakes and status assessed by probability approach among the urban adult population of Hyderabad city in South India

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Abstract

Purpose To assess the dietary inadequacies of micronutrients and the associated factors among the apparently healthy urban adults.

Methods This community-based cross-sectional study involved 300 urban adults (distributed into age groups: 21–40, 41–60, and > 60 years) residing in Hyderabad city, South India. Hemoglobin in whole blood, ferritin, folate, and vitamin B12 (B12) in plasma was estimated. Dietary intakes were assessed by three 24-h dietary recalls and calculated the probability of adequacy (PA) using estimated average requirement.

Results The prevalence of anemia (30%), iron deficiency (ID, 23%), and iron deficiency anemia (IDA, 14.3%) was independent of age but higher in women. While folate deficiency (32.2%) was independent of age and gender, B12 deficiency (35.5%) varied by both age and gender. The PA of iron (89%) was higher, while that of folate, B12, and zinc (1–11%) were noticeably low. The mean PA (MPA) across the ten micronutrients was 38%, independent of age and gender, but associated with the educational status. Energy intake was a strong predictor of the MPA. Cereals and millets predominantly contributed to the intake of thiamine, niacin, zinc, and iron; green leafy vegetables and fruits to vitamins A, C, folate, and iron; animal foods to B12; and milk and milk products to calcium, vitamin A, riboflavin, and B12. The unadjusted and adjusted logistic regression models revealed that micronutrient inadequacy was associated with greater risk of IDA and folate deficiency.

Conclusions These results indicate a higher prevalence of micronutrient deficiencies among the healthy urban adults possibly due to the inadequacy of multiple micronutrients.

Keywords Micronutrients · Dietary intake · Probability of adequacy · Mean probability of adequacy

Abbreviations

PA	Probability of adequacy
MPA	Mean probability of adequacy
EAR	Estimated average requirements
SD	Standard deviation
CV	Coefficient of variation
RDA	Recommended dietary allowance
NNMB	National Nutrition Monitoring Bureau
USDA	United States Department of Agriculture
IOM	Institute of Medicine
NAR	Nutrient adequacy ratio
B12	Vitamin B12
ID	Iron deficiency
IDA	Iron deficiency anemia
SES	Socio-economic status

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Introduction

Undernutrition and obesity continue to exist as a dual burden [1] which is hypothesized to be an integral part of the nutritional and socio-economic transition in developing countries like India [2]. The upsurge of micronutrient deficiency (hidden hunger) further adds to the current conundrum, thereby

contributing to non-communicable diseases (NCDs). With the rapid increase in economic development and urbanization clubbed with easy accessibility, the intake of refined foods has increased drastically even in low-income countries contributing significantly to the nutritional transition [3–5]. The National Sample Survey Organization (NSSO) observed a decrease in the intake of energy and protein and a simultaneous increase in the fat intake [6, 7]. Studies from the National Nutrition Monitoring Bureau (NNMB) have also reported a similar trend with a decline in the prevalence of underweight and a simultaneous increase in the overweight/obese population with high prevalence of micronutrient deficiencies among rural populations [8].

Micronutrients play a vital role in various biological functions and have implications for the regulation of key metabolic processes in the body. Micronutrient malnutrition, however, remains a grave nutritional concern which continues to be the most significant challenge in the face of human development, as it may have a marked effect on the overall health of the individual and in turn impact the society [9]. Deficiency of micronutrients is linked to a wide range of adverse health outcomes, such as birth defects, growth retardation, impaired cognition, and mental development leading to a plethora of chronic degenerative diseases with increased morbidities and mortalities. Micronutrient deficiency is primarily due to inadequate dietary intakes, poor food quality, and minimal dietary diversity [10, 11], but the extent of deficiencies varies depending on the economic and social factors, varied cultural habits and values, ignorance due to high female illiteracy, isolation, and stress [12]. In under-developed countries, systemic infections and parasitic diseases further aggravate the micronutrient deficiencies by reducing nutrient absorption and biological utilization [13].

The micronutrient status among populations can be measured indirectly through dietary assessment, or it can be directly measured by determining the nutrient content in body fluids or tissues. The dietary assessment would be a simple non-invasive tool for assessing the risk of low micronutrient status both at the individual and population level. Assessment of micronutrient deficiencies is helpful in the development of appropriate intervention strategies specific to geographical areas, age groups, populations, and also for monitoring and evaluation of programs to improve the nutritional status of vulnerable populations. Globally, epidemiological studies have focused on a select few micronutrients (vitamin A, iodine, and iron) owing to their widespread prevalence, particularly in vulnerable groups such as women, children, and adolescents [14]. In a previous study, we assessed the plasma concentrations and the dietary intakes of vitamin B12 (B12) and folate in the adult population and found that only 40% of the adult population was meeting 70% of the recommended dietary allowance (RDA) of B12, which is consistent with the high prevalence of its biochemical deficiency [15]. These findings prompted us to extend the study to other micronutrients of public health interest which includes thiamine, riboflavin, niacin, vitamin A, vitamin C, zinc, calcium, and iron in the apparently healthy adult groups. Furthermore, the probability of adequacy (PA) approach using estimated average requirement (EAR) is considered more relevant than the ratios of intakes to the RDA (which exceeds the requirements of 97.5% of the population), as it is based on a comparison of two distributions—nutrient requirement and nutrient usual intake [16]. Hence, in the present study, we employed PA to assess the dietary adequacy of micronutrients along with the biochemical status of micronutrients and the associated factors among the urban South Indian adult population.

Methodology

Study design

A community-based cross-sectional study was conducted in an urban setup in Hyderabad city, Telangana State of India from October 2014 to September 2016. Hyderabad was selected as the study area, because it is a Metro city and is expected to represent various cultures and ethnicity of Indians. To represent the population from different socio-economic backgrounds, Hyderabad city was stratified into four zones (East, West, North, and South) and further subdivided into 150 wards. Two wards from each zone were selected by a simple random sampling procedure (random number table method).

Sample size

The sample size was estimated considering 40% of the adult population consumed adequate intake (> 70% RDA) of B12 [15]. Assuming 95% confidence interval (CI), a relative precision of 20%, and with a design effect of 2, the required sample size was 288.

Selection of study subjects

To enroll the subjects, health camps were organized at the randomly selected wards of Hyderabad city. A total of 300 apparently healthy subjects in the age group of 21–85 years: men ($n = 144$) and women ($n = 156$) who fulfilled the criteria were consented for participation (Fig. 1). The study subjects were arbitrarily categorized into three age groups viz., 21–40, 41–60, and > 60 years. Pregnant and lactating women were excluded from the study. All procedures involving human subjects were approved by the Institutional Ethics Committee (IEC; # CR9/I/2014) and followed the guidelines laid down in the 1964 Declaration of Helsinki and its later

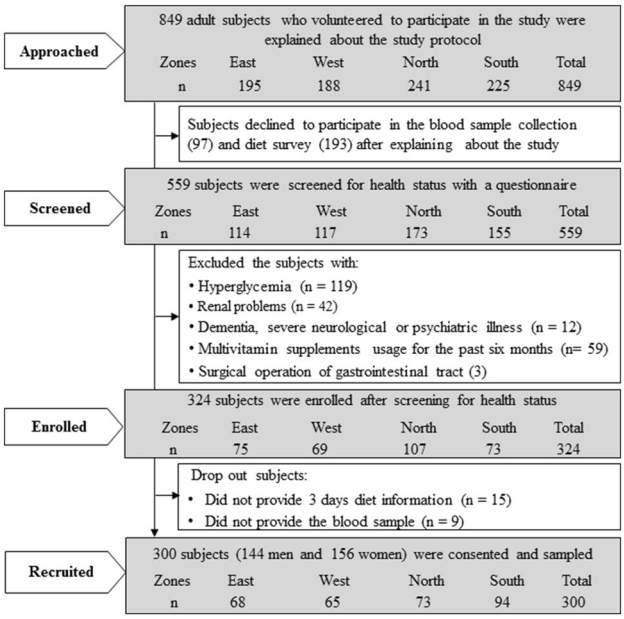

Fig. 1 Flowchart showing the details of the selection and recruitment of the study subjects

amendments. Written informed consent (or thumb impression in the case of uneducated) to participate in the study was obtained from the subjects.

Data collection

The subjects were interviewed by a trained interviewer [trained by the expert unit of NNMB at the National Institute of Nutrition (NIN)] to obtain information on socio-demographic parameters such as age, gender, education, type of family, employment status, and food habits. A family with a married couple and with or without their unmarried children is considered as nuclear family, a family with two or more married couples and with or without their children as joint family, and a family having a married couple (with or without their unmarried children) and their unmarried or widowed brothers or sisters, father, or mother as extended nuclear [17]. Subjects who never consumed animal foods (like poultry, mutton, eggs, and fish) were considered vegetarians, and the others were considered in the mixed diet group.

Anthropometric measurements

Height (precision to the nearest 0.1 cm) and body weight (precision to the nearest 0.1 kg) were measured using a calibrated anthropometric rod and SECA weighing scale, respectively [18]. Body mass index (BMI) was calculated as the ratio of weight in kilograms to the square of height in meters (kg/m^2). Individuals with BMI < 18.5 kg/m^2 were classified as underweight, 18.5–22.99 kg/m^2 as normal, 23–27.49 kg/m^2 as overweight, and ≥ 27.5 kg/m^2 as obese [19].

Biochemical estimations

Venous blood samples were collected in heparin tubes from the subjects early in the morning following an overnight fast. Blood samples were kept in ice packs and transported to the laboratory within 6 h after collection. Blood and plasma (separated by centrifugation at 3500 rpm/10 min) were aliquoted appropriately. Hemoglobin (Hb) analysis was carried out on the same day, and the plasma aliquots were stored in − 80 °C for the estimation of ferritin, C-reactive protein (CRP), B12, and folate. Hemoglobin was estimated in whole blood by the cyanmethemoglobin method using a spectrophotometer (Shimadzu UV 2600). Anemia was defined as Hb values < 13.0 g/dL in men and < 12.0 g/dL in women [20]. Ferritin was estimated using a solid-phase enzyme-linked immunosorbent sandwich assay (ELISA) method (# FR248T, Calbiotech, USA). C-reactive protein was determined by Duo Set ELISA development system (# DY1707, R&D System, Minneapolis, USA) according to the manufacturer's protocol. The ferritin values were excluded for the one, whose CRP concentrations are > 5 mg/L (in 7 samples out of total 300). Subjects having ferritin concentrations < 15 µg/L [21] were considered as iron deficient (ID) and ferritin concentrations < 15 µg/L along with anemia (< 13.0 g/dL in men, < 12.0 g/dL in women) were considered as iron deficiency anemia (IDA) [22]. Plasma concentrations of B12 and folate were determined by radioimmunoassay (RIA) method using a dual-count solid-phase no-boil RIA kit (Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) [23]. Radioactivity was measured using a gamma counter with a dual channel for determining ^{57}Co (B12) and ^{125}I (folate) simultaneously (Perkin Elmer, 3wizard 1480, USA). The concentrations of B12 < 150 pmol/L and folate < 10 nmol/L were considered as deficient [24]. A set of three in-house quality control samples were analyzed along with each set of samples for all the analytes, and the inter/intra-assay variation was always < 7%.

Dietary assessment

The 24-h dietary recall was conducted by a face-to-face interview on three different days (2 non-consecutive weekdays and one weekend day) to carry out the diet survey ($n = 300$) [25]. The person who cooked food for the entire household was interviewed for dietary intake information of the previous day. A set of standardized 12 cups and two spoons [26] were used to obtain diet intake information of cooked foods, which aided subjects to visually estimate the portion sizes. A portable electronic digital diet scale

(SecaCulina 852®) with 1 g accuracy was used for direct weighing of the staple raw foods. The foods that were consumed but currently not in-stock at the household were quantified for the edible portion of raw foods in the metabolic kitchen (at NIN), and the corresponding values were used in the calculations. The quantities of raw foods were computed from the intakes of cooked foods [Intakes of the raw food by the individual = (Quantity of raw food in the preparation/Total cooked quantity of food) × Individual intake of cooked food]. The nutritive values of raw foods were taken from the Nutritive Value of Indian Foods (NVIF) [27], while United States Department of Agriculture (USDA) [28] food and the nutrient database was used for those foods that did not have a nutrient value in NVIF. To ensure minimum variation between the NVIF and the USDA database, the nutritive values of some common foods were compared after correction for moisture values, and the variations were found to be comparable in the range of 10–20%. Daily individual consumption of nutrients was calculated by taking the average of 3 days of diet survey. The total daily consumption was computed based on the above-mentioned nutritive value databases, using the in-house software.

Probability of adequacy

The adequacy of micronutrients was assessed using the probability approach which relates an individual's usual intake of nutrients to the distribution of requirements for a particular life stage and gender group using EAR values and its standard deviation (SD) [16, 29–31]. The probability approach plots each individual's intake data from the study population and constructs a risk curve using the requirement (EAR and SD) distribution of the group [Z score = (Intake − EAR)/SD of the requirement]. The risk curve specifies the probability that the given intake is inadequate for the individual consuming that nutrient. Then, the risk curve was compared to the distribution of usual intakes of the study population to determine what proportion of the population has an inadequate intake. Probability approach is based on the two key assumptions: (1) intakes and requirements are independent and (2) the distribution of requirements is known [32, 33]. Thus, the PA determines the probability that an individual's intake in a group meets the requirements, and then, their mean of the individual probabilities is obtained, which is used to estimate the prevalence of adequacy of a particular nutrient. The PA was computed using the "CDFNORM" function in SPSS software. Hence, the micronutrient adequacy was evaluated by calculating the PA for ten micronutrients in this study: calcium, vitamin A, thiamine, riboflavin, niacin, vitamin C, B12, zinc, folate, and iron which are of public health importance. The recommended EAR, as set by the Institute of Medicine (IOM) (National Academies, Food and Nutrition Board) [34], according to sex and age group was considered for the calculation of PA. The SD of requirements was calculated using the coefficient of variation (CV) of requirements [SD = CV × EAR]; the CVs were 20% for vitamin A, 10% for calcium [35], thiamine, riboflavin, vitamin C, B12, folate, iron, and zinc, and 15% for niacin. Bioavailability of iron and zinc was assumed to be 5%. The resulting value for PA ranged from 0 to 100%, and an overall mean PA (MPA) was calculated by averaging the PA across the ten nutrients. The prevalence of inadequacy was defined by considering MPA below 50% (MPA < 0.5) [34, 36, 37].

The contribution of food groups

The foods were divided into 12 food groups, namely: cereals and millets, pulses and legumes, green leafy vegetables, roots and tubers, other vegetables, nuts and oilseeds, spices and condiments, fruits, animal foods, milk and milk products, fats, and oils and sugars. The contribution of the particular food group to the micronutrient intake was calculated as [(Raw intake of an individual for a particular food × micronutrient content of particular food per 100 g)/100]. Likewise, the individual intake of the particular micronutrient from all the foods (rice, wheat, jowar, rice flakes, puffed rice, etc.) that belong to the cereals and millets food group was summed up. Then, the summed value of that particular nutrient of all the individuals was averaged, which is reported.

Statistical analyses

Data analyses were performed using the software package SPSS (version 19.0, SPSS Inc, Chicago, IL, USA). As most of the data were skewed, the intake of food groups and nutrients among the age groups and between the genders was reported as medians, 25th (P_{25}) and 75th (P_{75}) percentiles, and comparisons for the same were carried out by Kruskal–Wallis test and Mann–Whitney U test, respectively. Comparison of mean values of the variables across the age groups was done by one-way ANOVA with a least significant difference (LSD) multiple comparisons and between the genders by student's t test. The Chi-square (χ^2) test was used for testing the association between categorical variables. Multivariate logistic regression analysis was performed to identify factors associated with inadequacy of nutrients (MPA < 0.5) and also to examine the association of inadequacy (MPA < 0.5) with anemia, ID, IDA, and deficiency of folate and B12. The level of significance was considered at $p < 0.05$.

Results

Socio-demographic characteristics of the study subjects

The gender distribution was almost similar in all the age groups. Majority of the subjects were baccalaureate graduates and above (57%) and were consuming mixed diets (75.7%). About 6% of the subjects were underweight, 31.3% had a normal BMI, 42.7% were overweight, and 20% were obese (Table 1).

Table 1 Socio-demographic characteristics ($n = 300$)

Variable	*n* (%)
Age (years)	
21–40	101 (33.7)
41–60	104 (34.7)
> 60	95 (31.6)
Gender	
Men	144 (48.0)
Women	156 (52.0)
Religion	
Hindu	239 (79.7)
Muslim	32 (10.6)
Others	29 (9.7)
Type of family	
Nuclear	210 (70.0)
Extended nuclear	37 (12.3)
Joint	53 (17.7)
Literacy	
Uneducated	32 (10.7)
Up to 12th standard	97 (32.3)
Graduation and above	171 (57.0)
Occupation	
Service	95 (31.7)
Business	5 (1.6)
Home maker	120 (40.0)
Retired	72 (24.0)
Others	8 (2.7)
Food pattern	
Vegetarian	73 (24.3)
Mixed diet	227 (75.7)
BMI (kg/m^2)	
< 18.5	18 (6.0)
18.5–22.99	94 (31.3)
23–27.49	128 (42.7)
≥ 27.5	60 (20.0)

Values represent *n* (%)

BMI body mass index

Biochemical parameters

While the mean Hb levels were significantly different among the age groups ($p = 0.004$), the ferritin levels were comparable ($p = 0.090$) (Table 2). The prevalence of anemia (30%), ID (23%), and IDA (14.3%) was independent of age, but was higher in women (Table 3). Mean plasma concentrations of folate ($p = 0.047$) and B12 ($p = 0.002$) were significantly different between the age groups (Table 2). While folate deficiency (32.2%) was independent of age and gender, B12 deficiency (35.5%) varied by both age and gender (Table 3).

Dietary intake of food groups and nutrients

The median intake of vegetables ($p < 0.001$), roots and tubers ($p < 0.001$), spices and condiments ($p < 0.001$), animal foods ($p < 0.001$), milk and milk products ($p < 0.001$), and sugars ($p = 0.003$) was significantly different among the age groups (Table 4). The dietary intake of calcium ($p < 0.001$) and riboflavin ($p < 0.001$) was significantly high in the > 60 age group. The 41–60 age group had significantly lower intakes of B12 ($p = 0.019$) (Table 4). The intakes of the majority of food groups and nutrients were lower in women compared to men for the given age groups (Supplementary Table 1). The median intake of food groups such as cereals and millets, roots and tubers, spices and condiments, and animal foods, and the nutrient intakes of energy, vitamin A, thiamine, riboflavin, niacin, B12, zinc, folate, and iron were significantly lower in women than men ($p < 0.05$) (Supplementary Table 2).

Probability of adequacy among the age groups and between genders

The PA is lowest for folate (1%) followed by B12 (6%), zinc (11%), riboflavin (37%), niacin (40%), calcium (42%), vitamin A (43%), vitamin C (52%), thiamine (58%), and iron (89%). The MPA across the ten micronutrients was 38% (Table 5). The PA of riboflavin was significantly lower in the 21–40 age group ($p = 0.004$), and that of zinc ($p = 0.049$) was significantly lower in the > 60 age group compared to the other two respective groups, while the MPA was comparable ($p > 0.05$) among the age groups (Table 5). In women, PA of calcium ($p = 0.002$), niacin ($p = 0.005$), folate ($p = 0.019$), and iron (< 0.001) was significantly lower, whereas that of riboflavin ($p = 0.004$), and zinc ($p < 0.001$) was higher compared to men (Table 5). Between the genders, the MPA was similar ($p = 0.484$). The risk of micronutrient inadequacy (MPA < 0.5) was about 68% in the study subjects and was not associated with age groups ($p > 0.05$) (Fig. 2). Energy intake was positively associated with MPA ($r^2 = 0.364$, $p < 0.001$) (Fig. 3). The PA of calcium, thiamine, niacin, folate, and iron is low in women, whereas

Table 2 Plasma concentrations of clinical parameters and vitamins among different age groups and between genders

Parameters	21–40 years ($n=101$) Mean (SE) Median (P_{25}–P_{75})	41–60 years ($n=104$) Mean (SE) Median (P_{25}–P_{75})	> 60 years ($n=95$) Mean (SE) Median (P_{25}–P_{75})	*p* value	Men ($n=144$) Mean (SE) Median (P_{25}–P_{75})	Women ($n=156$) Mean (SE) Median (P_{25}–P_{75})	Pooled ($n=300$) Mean (SE) Median (P_{25}–P_{75})	*p* value
Hb (g/dL)	13.1[ab] (0.2)	12.6[a] (0.1)	13.6[ab] (0.2)	0.004**	14.3[a] (0.1)	11.9[b] (0.1)	13.1 (0.1)	< 0.001**
	13.4 (11.7–14.7)	12.5 (11.8–13.6)	13.5 (12.1–15.0)		14.4 (13.6–15.3)	12.0 (11.4–12.6)	13.0 (11.8–14.4)	
Ferritin (μg/L)	45.6[a] (5.2)	70.8[a] (8.5)	56.7[a] (8.6)	0.090	84.5[a] (7.5)	31.4[b] (3.0)	57.6 (4.4)	< 0.001**
	33.2 (8.5–69.5)	54.5 (17.5–108.3)	35.5 (16.5–74.5)		62.6 (33.9–110.4)	20.7 (8.9–45.8)	37.6 (15.9–79.0)	
Folate (nmol/L)	13.4[a] (0.8)	16.1[b] (0.8)	13.6[a] (1.0)	0.047*	12.7[a] (0.6)	15.9[b] (0.8)	14.4 (0.5)	< 0.001**
	12.2 (9.3–15.0)	14.0 (9.5–20.4)	10.9 (7.8–17.2)		11.2 (8.3–16.1)	13.7 (9.7–19.5)	12.7 (9.1–17.4)	
B12 (pmol/L)	206.6[a] (14.8)	218.2[a] (14.9)	290.8[b] (22.1)	0.002**	196.9[a] (11.8)	269.1[b] (15.3)	235.2 (10.0)	< 0.001**
	169.7 (118.1–236.2)	177.1 (120.3–273.1)	247.2 (147.6–357.9)		160.5 (114.2–247.2)	225.1 (147.6–332.1)	188.2 (125.5–298.9)	

Mean values across age groups were compared by one-way ANOVA with LSD multiple comparisons and between the genders by student's *t* test

Significant differences of mean values between the age groups ($p<0.01$, $p<0.05$) and genders ($p<0.01$) are indicated by different superscript letters a,b

Values are means (SE) and medians (P_{25}, 25th percentile; P_{75}, 75th percentile)

Hb hemoglobin

**Significantly different at $p<0.01$. *Significantly different at $p<0.05$

Table 3 Prevalence of anemia, iron deficiency, iron deficiency anemia, and deficiency of folate and B12 among different age groups and between genders

Parameter	21–40 years ($n=101$)	41–60 years ($n=104$)	> 60 years ($n=95$)	*p* value	Men ($n=144$)	Women ($n=n=156$)	Pooled ($n=300$)	*p* value
Anemia	27.1[a]	37.6[a]	24.3[a]	0.129	10.4[a]	47.8[b]	30.0	< 0.001**
ID	30.4[a]	19.7[a]	17.7[a]	0.196	9.3[a]	36.0[b]	22.8	< 0.001**
IDA	20.3[a]	12.1[a]	9.8[a]	0.182	5.2[a]	23.2[b]	14.3	< 0.001**
Folate deficiency	32.0[a]	29.3[a]	36.4[a]	0.608	37.2[a]	27.8[a]	32.2	0.112
B12 deficiency	41.2[a]	39.4[a]	23.4[b]	0.030*	45.7[a]	26.4[b]	35.5	< 0.001**

Data represent % deficiency

Significant differences of mean values between the age groups ($p<0.05$) and genders ($p<0.01$) are indicated by different superscript letters a,b

ID iron deficiency anemia–ferritin concentrations < 15 μg/L, *IDA* iron deficiency anemia–ferritin concentrations < 15 μg/L along with anemia (< 13.0 g/dL in men, < 12.0 g/dL in women)

**Significantly different at $p<0.01$. *Significantly different at $p<0.05$

vitamin A, riboflavin, and zinc were low in men across all the age groups (Supplementary Table 3).

Contribution of different food groups to micronutrient intake

The contribution of different food groups to micronutrient intakes is shown in Table 6. Cereals and millets contribute to 39.2% of thiamine, 25.1% of riboflavin, 62.1% of niacin, 40.4% of zinc, 20.8% of folate, and 35.3% of iron. Pulses and legumes contributed 16.2% of thiamine and 19.3% of folate. Green leafy vegetables contributed 27.2% of vitamin A, 15.1% of vitamin C, 20.0% of folate, and 12.2% of iron. Fruits contributed 21.0% of vitamin A and 41.7% of vitamin C. Animal foods contributed 22.3% of B12. Milk and milk products contributed to calcium (59.6%),

Table 4 Median (P_{25}–P_{75}) intake of food groups and nutrients among different age groups

Food groups/nutrients	21–40 years ($n=101$) Median (P_{25}–P_{75})	41–60 years ($n=104$) Median (P_{25}–P_{75})	>60 years ($n=95$) Median (P_{25}–P_{75})	Pooled ($n=300$) Median (P_{25}–P_{75})	p value
Food groups					
Cereals and millets (g)	270.1[a] (222.8–328.7)	253.5[a] (219.2–316.8)	249.6[a] (209.5–290.9)	261.5 (219.7–320.6)	0.190
Pulses and legumes (g)	26.5[a] (12.0–52.9)	38.5[a] (21.6–56.4)	36.0[a] (17.4–56.0)	34.7 (16.7–55.7)	0.078
Green leafy vegetables (g)	12.7[a] (4.3–28.0)	15.9[a] (4.9–29.3)	15.5[a] (3.5–37.0)	15.2 (4.3–30.3)	0.541
Other vegetables (g)	41.0[a] (17.1–71.9)	60.6[a] (24.0–94.1)	78.0[b] (46.3–114.2)	56.5 (25.1–94.0)	<0.001**
Roots and tubers (g)	81.2[a] (55.4–119)	74.2[a] (40–101)	50.1[b] (23.5–87.0)	69.5 (37.1–102.8)	<0.001**
Nuts and oil seeds (g)	6.1[a] (1.9–13.6)	7.9[a] (2.8–16.5)	4.8[a] (0.87–17.2)	6.4 (1.6–16.4)	0.120
Spices and condiments (g)	11.3[a] (8.9–15.2)	12.4[a] (9.2–16.9)	9.4[b] (6.2–11.8)	11.1 (8.4–14.5)	<0.001**
Fruits (g)	111.1[a] (65.7–200.1)	114.3[a] (67.4–174.3)	102.7[a] (51.1–167.9)	112.3 (60.6–177.7)	0.585
Animal foods (g)	34.6[a] (12.6–69.3)	10.5[b] (0.0–50.7)	0[b] (0.0–30.9)	16.3 (0.0–50.1)	<0.001**
Milk and milk products (g/mL)	203.1[a] (119.6–300)	245[b] (171.9–365)	346.9[c] (284.5–405.2)	271.1 (176.7–368.1)	<0.001**
Fats and oils (g)	17.7[a] (10.0–27.0)	18.5[a] (8.9–29.2)	16.4[a] (7.7–27.3)	17.6 (8.9–27.9)	0.819
Sugars (g)	11.3[a] (7.5–16.0)	11.4[a] (7.5–16.2)	9.3[b] (4–12.8)	10.5 (6.2–15.0)	0.003**
Nutrients					
Energy (Kcal)	1924 (1686–2359)	1938 (1665–2258)	1865 (1561–2092)	1919 (1665–2240)	0.152
Calcium (mg)	669.4[a] (554.9–919.7)	809.8[b] (626.9–1054.9)	873.3[b] (764.7–1007.5)	797.0 (629.0–991.5)	<0.001**
Vitamin A (µg)	508.4[a] (367.6–689.3)	509.7[a] (342.6–655.5)	465.6[a] (298.1–715.6)	503.3 (342.8–688.5)	0.731
Thiamine (mg)	1.03[a] (0.77–1.17)	1.03[a] (0.8–1.3)	1.0[a] (0.77–1.3)	1.03 (0.77–1.3)	0.788
Riboflavin (mg)	0.83[a] (0.67–1.03)	0.87[a] (0.7–1.1)	1.02[b] (0.83–1.2)	0.90 (0.73–1.07)	<0.001**
Niacin (mg)	10.8[a] (8.0–13.2)	10.3[a] (8.9–12.9)	10.1[a] (8.3–12.6)	10.4 (8.5–12.8)	0.608
Vitamin C (mg)	70.7[a] (43.3–102.6)	72.7[a] (45.9–105.3)	58.7[a] (41.8–103.3)	66.4 (42.9–104.5)	0.452
B12 (µg)	0.77[a] (0.43–1.13)	0.5[b] (0.33–0.77)	0.57[ab] (0.43–0.83)	0.57 (0.4–0.95)	0.019*
Zinc (mg)	6.1[a] (4.7–7.1)	5.7[a] (4.9–6.8)	5.6[a] (4.8–6.6)	5.7 (4.9–6.8)	0.305
Folate (µg)	151.9[a] (116.4–199.7)	162.3[a] (130.5–198.6)	167.4[a] (128.1–216.4)	157.7 (123.2–201.0)	0.171
Iron (mg)	11.2[a] (8.7–15.0)	11.7[a] (9.3–14.4)	10.2[a] (7.8–14.1)	11.2 (8.3–14.4)	0.142

Values represent medians and 25th and 75th percentiles (P_{25}–P_{75})

Significant differences ($p<0.01$, $p<0.05$) of median values between the groups are indicated by different superscript letters a, b, c.

**Significantly different at $p<0.01$. *Significantly different at $p<0.05$

vitamin A (32%), riboflavin (40.5%), B12 (75.7%), and zinc (18.3%).

Factors associated with micronutrient inadequacy

The risk of micronutrient inadequacy and its association with different factors were compared by calculating the odds ratios with unadjusted, adjusted for age as a continuous variable and gender (model 1) and adjusted for age as a continuous variable, gender and energy (model 2) logistic regression models. The odds of micronutrient inadequacy was higher in uneducated subjects (OR 4.40; 95% CI 1.46, 13.3) and the high school group (OR 3.01; 95% CI 1.65, 5.52) compared to graduation and above and it remained statistically significant in adjusted models (Supplementary Table 4). Similarly, subjects with micronutrient inadequacy (<0.5) had a higher risk of IDA (OR 5.51; 95% CI 1.26, 24.1), and folate deficiency (OR 2.26; 95% CI 1.24, 4.11) and the associations remained statistically significant after adjusting for age, gender, and energy (Supplementary Table 5).

Discussion

Micronutrient deficiencies referred to as hidden hunger, mainly due to dietary inadequacies, are not apparent but considered ubiquitous affecting more than 2 billion people globally and one-third of them are residing in India [38]. Deficiency of micronutrients has been an unrelenting problem throughout the developing world especially in southern Asia contributing to exorbitantly high mortality and morbidity. Thus, knowledge of the prevalence of micronutrient deficiencies/inadequacies and associated factors such as dietary intake and non-dietary factors like age, lifestyle, environment, genetics, and socio-economic conditions will be crucial in the development of appropriate intervention strategies for their prevention and treatment.

Table 5 Probability of adequacy and MPA among different age groups and between the genders

Nutrients	21–40 years ($n=101$)	41–60 years ($n=104$)	>60 years ($n=95$)	p value	Men ($n=144$)	Women ($n=156$)	p value	Pooled ($n=300$)
PA (%)								
Calcium (mg)	36[a]	43[a]	46[a]	0.261	50[a]	34[b]	0.002**	42
Vitamin A (µg)	44[a]	42[a]	41[a]	0.881	39[a]	46[a]	0.155	43
Thiamine (mg)	55[a]	62[a]	57[a]	0.482	62[a]	54[a]	0.134	58
Riboflavin (mg)	29[a]	35[a]	49[b]	0.004**	30[a]	44[b]	0.004**	37
Niacin (mg)	42[a]	40[a]	36[a]	0.505	46[a]	34[b]	0.005**	40
Vitamin C (mg)	53[a]	57[a]	44[a]	0.178	48[a]	55[a]	0.204	52
B12 (µg)	9[a]	5[a]	4[a]	0.260	9[a]	4[a]	0.115	6
Zinc (mg)	14[a]	13[a]	6[b]	0.049*	6[a]	16[b]	<0.001**	11
Folate (µg)	2[a]	1[a]	2[a]	0.813	3[a]	1[b]	0.019*	1
Iron (mg)	85[a]	90[a]	92[a]	0.193	95[a]	83[b]	<0.001**	89
MPA (%)	37[a]	39[a]	38[a]	0.790	39[a]	37[a]	0.484	38

Values represent percentages. Mean values across the age groups were compared by one-way ANOVA with LSD multiple comparisons. Mean values between the genders were compared by student's t test

Significant differences ($p<0.01$, $p<0.05$) of mean values among the age groups and between the genders are indicated by different superscript letters a,b

PA probability of adequacy, *MPA* mean probability of adequacy

**Significantly different at $p<0.01$. *Significantly different at $p<0.05$

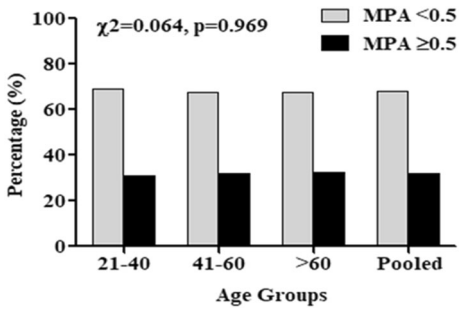

Fig. 2 Mean probability of adequacy (MPA) among the different age groups. Pooled data represent the total number of samples ($n=300$). Data represent % inadequacy (<0.5) and % adequacy (≥0.5) of micronutrients. $p<0.05$ was considered to be significant

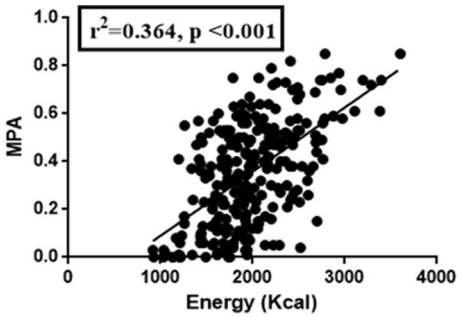

Fig. 3 Association between energy and mean probability of adequacy (MPA). Pooled data represent the total number of samples ($n=300$)

Dietary intake is considered to be one of the major determinants of the micronutrient status of individuals. Nutrient adequacy of an individual's diet and its contribution towards maintaining adequate micronutrient status are assessed by different approaches such as (1) the probability approach, (2) the EAR cut-point method, and (3) nutrient adequacy ratio (NAR) to name a few. These approaches majorly depend on the subjects (individual or population), the target nutrient, and the type of intake distribution [39]. The probability approach and the EAR cut-point methods compare the individuals'/populations' nutrient intake with their requirement. The probability approach method is based on the estimation of the probability of inadequate intakes for each individual in a population, averaging the probabilities, and then using this average as an estimate of the prevalence of inadequacy. This method assumes that the correlation between intake and requirement is low and that the distribution of requirements is known. The EAR cut-point method is a shortcut method of the probability approach, and it measures the prevalence of inadequate intakes in terms of the proportion of the population with usual intakes below the EAR. However, the EAR cut-point method follows the assumption of symmetrical distribution of the requirements, hence, is not suitable for some nutrients such as iron (as the requirement distribution of iron is known to be highly skewed) [32]. EAR cut-point method-100 is reported to overestimate when compared to probability approach [40]. In the third approach, NAR is

Table 6 Contribution of different food groups to micronutrient intakes

Food groups	Calcium (mg)	Vitamin A (µg)	Thiamine (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin C (mg)	B12 (µg)	Zinc (mg)	Folate (µg)	Iron (mg)
Cereals and millets (g)	7.1	1.4	39.2	25.1	62.1	0.1	0.15	40.4	20.8	35.3
Pulses and legumes (g)	4.0	2.2	16.2	7.9	8.1	0.2	0.05	11.2	19.3	12.1
Green leafy vegetables (g)	4.5	27.2	1.3	4.4	1.1	15.1	0.3	0.05	20.0	12.2
Other vegetables (g)	2.1	2.5	3.5	3.8	2.5	13.1	0.06	8.9	6.4	5.2
Roots and tubers (g)	2.9	5.2	4.7	0.6	4.4	10.9	0.05	3.0	2.0	5.0
Nuts and oil seeds (g)	3.0	0.8	3.1	0.7	6.9	0.3	0.1	5.1	1.2	3.5
Spices and condiments (g)	4.9	2.4	4.1	3.2	3.9	9.3	0.004	3.9	0.7	5.7
Fruits (g)	7.0	21.0	9.7	8.6	3.8	41.7	0.01	4.4	10.4	9.8
Animal foods (g)	2.9	4.0	2.1	4.9	2.0	0.1	22.3	5.4	3.5	3.7
Milk and milk products (g/mL)	59.6	32.0	15.5	40.5	4.7	9.5	75.7	18.3	17.0	6.9
Fats and oils (g)	1.1	0.9	0.02	0.03	0.01	0.004	0.3	0.01	0.07	0.1
Sugars (g)	0.7	0.001	0.001	0.02	0.02	0.001	0.002	0.004	0.002	0.4

Values are in percentages

an index of nutrient adequacy, which compares the individual's daily intake of a nutrient with the RDA for that nutrient, and mean adequacy ratio (MAR) is the sum of NAR divided by the total number of selected nutrients [41]. However, the probability approach using EAR is considered more relevant than the ratios of intakes to the RDA (which exceeds the requirements of 97.5% of the population), as it is based on a comparison of two distributions—nutrient requirement and nutrient usual intake [16]. We employed a probability approach method considering its strength in assessing the nutrient adequacy of individuals. To acquire a holistic approach of micronutrient inadequacy in urban adults, MPA for ten micronutrients that are of public health importance was assessed.

An MPA of 38% was observed across ten micronutrients among the study subjects which was similar to the previously reported study among women of reproductive age in urban Burkina Faso (West Africa) with an MPA of 38% for 11 micronutrients [36]. Another study reported an MPA of 67% in men and 58% in women for 15 micronutrients in a Continuing Survey of Food Intakes by Individuals (CSFII) cohort conducted by USDA [16]. A cross-sectional study among Tehranian (Iran) women aged 18–80 years reported the MPA of 50% across 14 micronutrients [42]. Two studies from Haryana (India) reported MPA for five micronutrients as 57% in rural and 49% in the urban adolescents [43] and in rural children (40%) [44]. Whereas the majority of the studies from India mainly assessed the dietary intake deficits with reference to RDA [45], and a direct comparison of these studies was not possible. Energy intake in the present study was a strong predictor of the MPA as energy intake plus four non-dietary variables (age, BMI, occupation, and education) explained 36% of the variance in the mean probability of nutrient adequacy. Similarly, a study in Iranian women and another study of CSFII cohort by USDA reported that the energy intake explained 53% and 54–60% of the variance in the MPA, respectively [16, 42].

The prevalence of anemia was found to be 30% with higher prevalence in women (48%) compared to men (10%), and is comparable to the reported prevalence of anemia among women (55%) and men (10%) in the state of Telangana [46]. Poor iron density in foods coupled with its low bioavailability is the major etiological factor for the wide spread prevalence of anemia in India [47, 48]. However, a low iron inadequacy (11%) was observed even after adjusting for 5% bioavailability, and this observation is in agreement with a low prevalence of ID (23%) measured by serum ferritin level. Furthermore, only 14.3% of the subjects had IDA, implies that only about 50% of the anemia is due to iron deficiency, and rest could be attributed to other factors including deficiency (inadequacy) of other micronutrients. This can also be explained by an association of micronutrient inadequacy and the risk of IDA even after adjustment

for age, gender, and energy. A previous study in 5–11 year school children found 12% IDA as opposed to 40% anemia [49]. Studies in young women of Bangalore, infants, and preschool children in the rural district of Telangana have demonstrated that iron deficiency is the predominant cause of anemia, and > 75% of anemic subjects had a concurrent iron deficiency [48, 50]. The apparent discrepancies in the prevalence of IDA among the reported studies could be due to the difference in age and socio-economic status (SES). Nevertheless, these studies together suggest that a significant proportion of anemia is independent of iron status and warrants further large-scale studies. Since iron supplementation is being practiced for treating anemia, considering the deleterious effects of iron, ascertaining the iron status may also, therefore, needed to guide the therapy.

In the current study, 32% and 35% of the subjects had sub-clinical folate and B12 deficiency. In agreement with these results, the remarkably higher dietary inadequacy of B12 (94%) and folate (99%) was observed in this population which may predispose them to chronic diseases. Previously, we have reported a higher prevalence of B12 deficiency in the lower age groups which correlated with their dietary intakes [15]. Deficiency of B12 is quite common in Indians and is mainly explained by a diet low in animal foods [51]. In the present study, the animal foods contributed about 22.3% and milk and milk products about 75.7% to B12 intake. However, the observed median intake of B12 is 0.57 µg per day which is much lower than the EAR (2.0 µg). Although cereals and millets, pulses and legumes, green leafy vegetables, and milk and milk products contributed to folate intake, the quantities were not sufficient to meet the requirements. This was elucidated by the association of micronutrient inadequacy and the risk of folate deficiency even after adjustment for age, gender, and energy. A study in North Indian urban slum and non-slum areas also reported markedly decreased dietary intakes of folate and B12, which are associated with hyperhomocysteinemia, as they are known to be integral players of the homocysteine metabolism [52] and implying an increased risk of cardiovascular diseases in Indians [53].

The inadequacy of zinc was 89% and higher in > 60 year age group. Cereals and millets, milk and milk products, and pulses and legumes mainly contributed to the zinc intake. Though 76% of the study subjects are in the mixed diet group, the animal foods contributed only 5.4% of zinc intake, which signifies the low consumption of animal foods. The dietary inadequacy of riboflavin, niacin, calcium, vitamin A, vitamin C, and thiamine was about 63%, 60%, 58%, 57%, 48%, and 42%, respectively. Men were more likely to have vitamin A, riboflavin, vitamin C, and zinc inadequacies when compared to women and is in line with a similar study [40]. The inadequacy of calcium was higher among women, which may be due to the lower intake of milk and milk products. Low-calcium intake is considered a risk factor for the development of osteoporosis, especially in postmenopausal women [54, 55]. The inadequacy of riboflavin was higher in 21–40 year age group because of low consumption of pulses and legumes, green leafy vegetables, and milk and milk products. Both in rural and urban areas, refined grains are preferred over whole grains and processing of rice and wheat might result in considerable losses of the micronutrients (especially thiamine and riboflavin), thus leading to their deficiencies [56]. Almost all the age groups are at risk of micronutrient inadequacy for one or the other micronutrient. One of the underlying factors of micronutrient inadequacy may be lower consumption of micronutrient-rich foods such as pulses, green leafy vegetables, fruits, and vegetables along with higher intakes of refined cereals, fats, and sugars that are poor sources of vitamins and minerals [57, 58]. Fruits and vegetables that provide a low-calorie micronutrient-rich diet with antioxidant properties have a protective effect against cardiovascular diseases [59–61] and other diet-related chronic diseases [62]. In the present study, the intake of fruits and vegetables was below the recommendations and contributed about 20–40% of the vitamins A and C.

The risk of micronutrient inadequacy was higher among the uneducated and high school groups even after adjustment for age, gender, and energy. Similar findings reported in other studies that the SES and illiteracy are the major determinants of micronutrient inadequacy [63]. Nutrition illiteracy was found to be a contributing factor for decreased consumption of fruits and vegetables in the state of Andhra Pradesh to which the current study location belonged to earlier [64]. A study showed that women of higher strata with respect to SES, education, and occupation have been consuming a healthier diet [65].

In conclusion, micronutrient inadequacy was observed in about 62% of the study population which is consolidated by the plasma levels of some micronutrients. The PA of folate, B12, and zinc wad noticeably low, probably explained by the low intakes of micronutrient-rich foods. While energy intake was a strong predictor of the MPA, micronutrient inadequacy was significantly associated with greater risk of IDA and folate deficiency, independent of age and gender, but associated with the educational status. Therefore, the MPA comprising of relatively more micronutrients than a selected few could be a good indicator of micronutrient adequacy. The findings highlight the need for necessary initiatives by the public health policy makers in achieving the recommended intakes from all food groups. Furthermore, nationwide representative data are needed for focused dietary and non-dietary interventions like education programs to improve peoples' decision-making capability regarding food choices, which in turn improves the diet quality. In addition, increasing dietary

diversity, particularly micronutrient-rich foods such as fruits and vegetables, flesh foods, while keeping the macronutrient content balanced, may improve micronutrient adequacy. Various other strategies like supplementation and fortification are also needed to prevent and alleviate micronutrient malnutrition.

Strengths and limitations

The strength of this paper lies in the fact that a standardized and validated dietary assessment protocol has been followed. Not only the dietary intake but also biochemical status of micronutrients was assessed to validate the determinants of risk of micronutrient inadequacy. The analysis of dietary intakes used the best methodology for calculating the intakes through the probability approach method. Though we used random sampling procedure, the subtle differences between the zones with different socioeconomic status cannot be completely ruled out. These findings reinforce our knowledge on the extent of micronutrient inadequacies and identify the factors that are related to their intakes which add value to the food and monitoring systems. Diet diversity score could not be calculated, as there was low variability in the number of food groups consumed by the study subjects. Furthermore, diet calculations used in the study do not account for cooking losses. Though the present study was conducted in a specific location in South India with small sample size, the findings might reflect the situation in this part of the country and indicate that such similar situation could possibly exist in other regions/areas. Nonetheless, further studies that are representative of the entire country needed to substantiate these findings.

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Compliance with ethical standards

Conflict of interest None of the authors has any conflict of interest.

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