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Status of Vitamin B12 and Folate among the Urban Adult Population in South India

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Key Words
Vitamin B12 · Folate · Diet survey · Adults · Community study

Abstract
Background: Deficiency of vitamin B12 (B12) and folate (FA) leads to a wide spectrum of disorders that affect all age groups. However, reports on B12 and FA status in healthy adults in India are limited. Hence, we determined the plasma levels and dietary intake of B12 and FA in the adult population. ***Methods:*** We conducted a community-based cross-sectional study in an urban setup among 630 apparently healthy adults distributed into 3 age groups: 21–40, 41–60 and >60 years. Plasma concentrations of B12 and FA were analyzed by radio immunoassay and dietary intake by 24-hour recall method. ***Results:*** The overall prevalence of FA deficiency was 12%, but there was no significant difference in plasma FA concentrations among the groups. While the overall prevalence of B12 deficiency was 35%, it was significantly higher in the 21–40 (44%) and 41–60 age groups (40%) when compared with the >60 group (30%). B12 deficiency was higher in vegetarians (54%) compared to those consuming mixed diet (31%), and the reverse was the case with FA. However, the dietary intakes of FA and B12 were not significantly different among the groups. ***Conclusions:*** These results indicate a higher prevalence of B12 deficiency in apparently healthy adults in an urban setup.

Introduction

Micronutrients, vitamin B12 (B12) and folate (FA), play a vital role in various biological functions and have implications in the regulation of various metabolic processes in the body [1, 2]. B12 is naturally available for human use only through ingestion of animal proteins, such as beef, poultry, fish, eggs and dairy products. FA is present in both animal and plant sources like liver, egg yolk, legumes and green leafy vegetables. Methylation of homocysteine (Hcys) to methionine and the synthesis of S-adenosylmethionine require these vitamins. FA encompasses many methylation reactions comprising DNA, proteins, phospholipids and neurotransmitter metabolism. B12 and FA have overlapping biological functions such as the synthesis of red blood cells, DNA and myelin sheath that are essential for normal growth and development. The crucial roles of B12 and FA in the healthy maintenance of skin, mucous membrane, hematological and central ner-

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vous system make them noteworthy. Deficiency of these vitamins can lead to a broad spectrum of neuropsychiatric disorders [3] that affect all age groups and that can often be reversed by early diagnosis and prompt treatment. Deficiency of B12 and FA increases the plasma concentration of Hcys, which is not only a risk factor for cardiovascular diseases but also the cause of megaloblastic anemia, macrocytosis and cognitive impairment in the elderly [4]. Both these vitamins are assessed together as they are involved in some common metabolic pathways; particularly, there is an inter-relationship between FA, B12 and methionine metabolism [5].

Although deficiency of these 2 vitamins can occur primarily as a result of insufficient dietary intake or poor absorption, various other factors such as sociocultural, gender, age, genetic and ethnic backgrounds are likely to influence their status [6–8]. Deficiency of B12 and FA, as assessed by the blood status, has been reported across population groups and in different stages of development in both developed and developing countries. In the global scenario, B12 and FA deficiencies have been associated with increased prevalence of metabolic complications such as diabetes mellitus [9], vascular diseases [10], neural tube defects [11], cardiovascular diseases [12], stroke [13], various types of cancers [14–16] and age-related macular degeneration [17].

In the Indian context, majority of the studies related to B12 and FA deficiencies are limited to some categories of the population such as elderly people, children and pregnant women. One study reported that despite a higher dietary intake of B12, the deficiency was found to be 16% in the normal South Indian urban elderly population [18]. Low cobalamin and FA concentrations in North Indian preschool children were associated with functional consequences [19]. Another study reported a low concentration of B12 but normal FA concentrations in North Indian adolescent population [20]. In a study conducted in the rural and urban populations of India, 67% of men were found to be B12 deficient, and vegetarians were at higher risk of deficiency [21]. In other cases, they are studied in connection with some disease states like cognitive dysfunction [22, 23], anemia [24], low birth weight [25], maternal malnutrition [26, 27] and hyperhomocysteinemia (HHcys) [28, 29], which once again revolves around the above said population. As a result, the apparently healthy population is seldom screened for B12 and FA concentrations.

Considering its contribution towards many metabolic disorders, the status of B12 and FA with regards to age and gender is critical. A comparative analysis of the same across different age groups is currently unavailable in India. In a different context, while assessing the status of B12 and Hcys in diabetic retinopathy, we found that the prevalence of B12 deficiency was 41%, but FA status was normal among healthy, non-diabetic middle-aged urban population with a mean age of 54 years [30], which prompted us to take up this study. Hence, in this study, we determined the prevalence of B12 and FA deficiencies in the general population and enumerated the dietary intake of these nutrients and its association with the plasma concentration.

Methods

Study Design, Subjects and Sample Collection

A community-based cross-sectional study was conducted predominantly in an urban setup during October 2012 to September 2014 in Hyderabad city and Khammam town of the Telangana state. The city and the town were divided into 4 zones (South Zone, East Zone, West Zone and North Zone). Furthermore, Hyderabad city was subdivided into 150 wards and Khammam town into 50 wards. Six wards were randomly selected to capture the entire population from both Hyderabad city and Khammam town. The study was approved by the Institutional Ethics Committee of National Institute of Nutrition and was carried out in conformity with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. After obtaining written consent from all the participants, venous blood samples were collected in heparin and ethylene diamine tetraacetic acid tubes in the morning following an overnight fast. The plasma was collected by centrifugation. The study population included 630 subjects: 347 men and 283 women aged 21–85 years, stratified into 3 age groups: 21–40, 41–60 and >60 years (elderly). Within the age groups, the distribution of men and women was approximately same. Apparently, healthy subjects were recruited, and those taking multivitamin supplements for the last 6 months or suffering from severe metabolic complications or having a history of surgical operation of the gastrointestinal tract or suffering from acute illness at the time of enrollment were excluded. History of diabetes or any other complication such as hypertension was also noted down.

Sample Size and Sampling Strategy

The sample size was calculated based on the prevalence of B12 deficiency as 30% [31]. Assuming 95% CI, 80% power and difference as 20%, the sample size arrived at 224 in each group. All the individuals who were aged above 20 years and willing to participate were included in the study.

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 and anthropometric rod, respectively. Body mass index (BMI) was calculated using the formula weight per height squared in kg/m^2.

Biochemical Estimations

Fasting blood sugar (FBS) was estimated in plasma by the glucose oxidase–peroxidase method using a kit (Biosystems, Barcelona, Spain). Glycosylated hemoglobin (HbA1c) was estimated in

Table 1. Clinical and demographic profile of study subjects

Parameter	21–40 years age group (n = 240) mean	SE	41–60 years age group (n = 184) mean	SE	>60 years age group (n = 206) mean	SE	F value	p value
Height, cm	163.38[a]	0.91	161.28[a]	0.59	160.0[b]	0.65	5.12	0.006
Weight, kg	64.62[a]	0.84	65.97[a]	0.87	64.34[a]	0.81	0.957	0.385
BMI, kg/m^2	23.97[a]	0.28	25.26[b]	0.34	25.21[b]	0.27	6.37	0.002
Hb, g/dl	13.44[a, b]	0.13	13.03[a]	0.15	13.54[b]	0.15	2.93	0.054
FBS, mg/dl	96.08[a]	1.24	118.25[b]	3.62	118.09[b]	2.81	28.50	0.000
HbA1c, %	6.29[a]	0.11	6.27[a]	0.1	7.18[b]	0.11	21.68	0.000
TC, mg/dl	151.13[a]	2.37	173.96[b]	2.86	173.14[b]	2.74	25.50	0.000
TG, mg/dl	94.7[a]	4.83	108.43[b]	4.62	108.43[b]	4.57	2.98	0.051
HDL, mg/dl	47.96[a]	1.6	41.26[a]	1.0	45.10[a]	1.38	0.70	0.493
LDL, mg/dl	97.27[a]	2.23	116.09[b]	5.82	110.69[b]	2.45	7.51	0.001
Plasma FA, ng/ml	6.3[a]	0.2	6.9[a]	0.26	6.5[a]	0.29	1.036	0.355
Plasma B12, pg/ml	258[a]	10.7	296[a]	13.7	365[b]	16.2	16.6	0.000
Dietary FA, μg/day	163.1[a] (n = 97)	6.2	167.7[a] (n = 99)	5.4	173.5[a] (n = 80)	6.6	0.576	0.631
Dietary B12, μg/day	1.31[a] (n = 97)	0.3	1.12[a] (n = 99)	0.42	0.73[a] (n = 80)	0.05	1.090	0.353
Hcys, μmol/l	17.4[a] (n = 89)	1.3	16.1[a] (n = 64)	0.9	18.0[a] (n = 73)	1.4	1.321	0.269

Values are mean and SE. Mean values across age groups were compared by one-way ANOVA 'F' test with post hoc test of Tukey's multiple comparisons. Significant differences ($p < 0.05$) of mean values among the age groups are indicated by different superscript letters (a, b).

whole blood by ion exchange chromatography using a kit (Biosystems) and hemoglobin (Hb) by the cyanmethemoglobin method. Lipid profile (high-density lipoprotein [HDL], total cholesterol [TC] and triglycerides [TG]) was analyzed using commercially available kits (Biosystems).

Estimation of Plasma B12, FA and Hcys

Plasma concentration of B12 and FA was determined by radio immunoassay (RIA) method using a dual-count solid-phase no-boil RIA kit (Siemens Medical Solutions Diagnostics, Los Angeles, Calif., USA) designed for simultaneous measurements of these vitamins [30]. Radioactivity was measured by the gamma counter with a dual channel for determining ^{57}Co (B12) and ^{125}I (FA) simultaneously (Perkin Elmer, 3 wizard 1480, USA). The concentrations of B12 <203 pg/ml and FA <3 ng/ml were considered deficient [32]. Plasma total Hcys was determined by employing a special reversed phase column for separating the analytes, supplied in the commercially available HPLC kit (Recipe Chemicals and Instruments GmbH, Germany).

Nutritional Assessment

Dietary intake was assessed in a sub-sample (n = 276, 127 men and 147 women) using systematic random sampling procedure. It was done by conducting a 3-day 24-hour recall method (2 non-consecutive weekdays and 1 weekend day) to capture intra and inter-individual variation, and average nutrient intake was calculated. The tools used for the same were validated. The nutritive values were taken as given in the Nutritive Value of Indian Foods (NVIF) [33, 34] and National Nutrition Monitoring Bureau database [35], whereas United States Department of Agriculture (USDA) food and nutrient database was used for those foods that did not have a nutrient value in NVIF [36]. To ensure minimum variation between the Indian and the USDA database, the nutritive values of some common foods after correction for moisture values were compared, and the variations were found to be comparable in the range of 10–20%. The total daily consumption was computed based upon the nutritive values according to the above-mentioned databases, and the calculations were done using an in-house software. The recommended dietary allowance (RDA) per the Indian standards for FA is 200 μg/day and for B12 is 1 μg/day [34]. Lacto-vegetarians were placed in the vegetarian group, whereas ovo-vegetarians and non-vegetarians were placed under the mixed diet group.

Statistical Analysis

Data were analyzed using the software package SPSS (Chicago, Ill., USA) for Windows, version 19.0. Median and interquartile ranges were calculated for skewed data, and comparisons for the same were carried out by the Kruskal–Wallis test. Mean and SE values of variables were calculated for normally distributed data. Comparison of mean values of these variables across the age groups was done by one-way analysis of variance (ANOVA) F test with post hoc test of Tukey's multiple comparisons. The chi-square test was used for comparison of the prevalence of B12 and FA deficiencies. The relationship between plasma B12 and FA with dietary B12 and FA was analyzed by the Spearman rank correlation coefficients. Linear regression was applied to examine the association between FA and B12 with Hcys. The level of significance was considered at p values <0.05.

Results

The characteristics of the subjects are shown in table 1. The gender distribution was almost the same in all the age groups (men and women were, respectively, 58 and 42%

Table 2. Plasma concentration and dietary intake of FA and B12

Age groups	Plasma concentration		Dietary intake	
	FA, ng/ml median (IQR)	B12, pg/ml median (IQR)	FA, μg/day median (IQR)	B12, μg/day median (IQR)
21–40 years	5.6[a] (4.1–7.2)	220[a] (150–320)	154.2[a] (116.9–201.0)	0.7[a] (0.4–1.1)
41–60 years	6.0[a] (4.3–8.6)	250[a] (160–380)	162.2[a] (130.5–198.6)	0.5[a] (0.3–0.7)
>60 years	5.2[a] (3.4–8.4)	300[b] (197–455)	167.4[a] (127.5–217.0)	0.5[a] (0.4–0.8)
p value	0.106	0.001	0.528	0.187

Values represent median and interquartile range (IQR). Significant differences ($p < 0.05$) of median values among the age groups are indicated by different superscript letters (a, b).

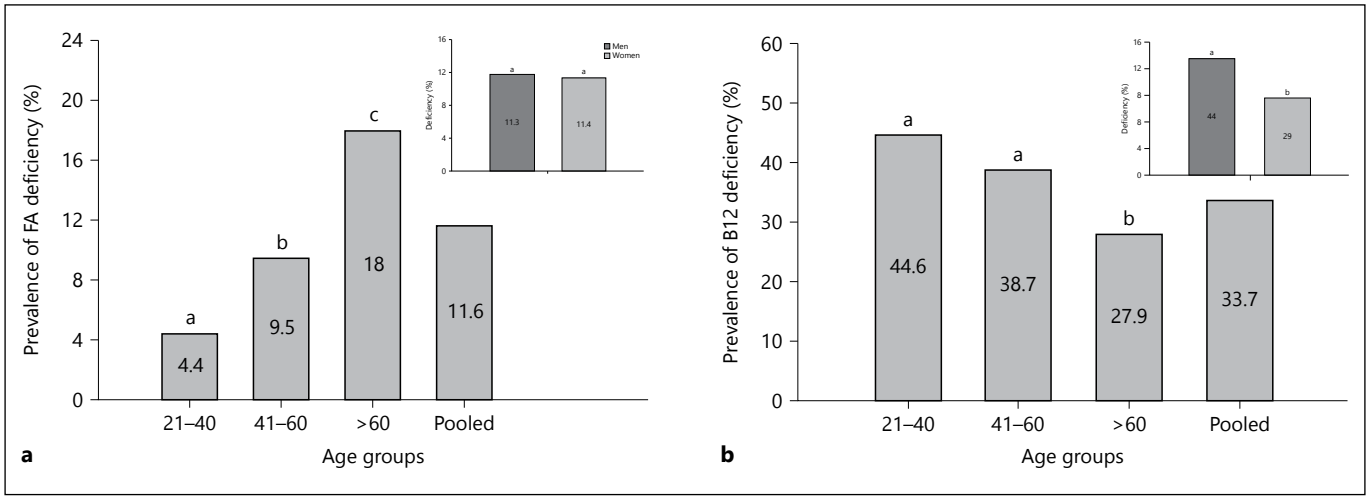

Fig. 1. Prevalence of FA (**a**) and B12 deficiency (**b**) among the different age groups. Pooled data represent the total number of samples (n = 630). Data represent % deficiency, and significant differences ($p < 0.05$) of mean values among the age groups are indicated by letters (a, b, c) above the bars. **Inset** in **a** and **b** shows the prevalence of respective vitamin deficiency between men and women.

in the 21–40, 52 and 48% in the 41–60 and 54 and 46% in the >60 years group). Though the mean values were statistically different, BMI and Hb were comparable among the age groups. While the FBS was significantly higher in the 41–60 and >60 years age groups when compared to the 21–40 age group, the HbA1c was higher in the >60 years group when compared to the 21–40 and 41–60 years age groups. However, plasma TC, TG and low-density lipoprotein were significantly higher in the 41–60 and >60 years age groups when compared to the younger (21–40 years) age group; HDL was comparable among all the age groups. The overall prevalence of anemia was found to be 28%, that is, the prevalence being higher (34%) in the 41–60 years age group compared to the 21–40 (24%) and 41–60 (27%) years age groups.

Median plasma FA concentrations were not significant among the age groups (table 2). The overall prevalence of FA deficiency was found to be 12%. However, the prevalence of FA deficiency (<3 ng/ml) was significantly higher in the >60 (18%) and 41–60 (10%) years age groups compared with the 21–40 years age group (5%; fig. 1a). The overall prevalence of B12 deficiency was found to be 34% (fig. 1b), which is higher when compared to the FA deficiency. Interestingly, median B12 concentrations of the 21–40 and 41–60 years age groups were significantly different from the >60 years age group (table 2). In concurrence with the (median) plasma data, the prevalence of B12 deficiency (<203 pg/ml) was significantly higher in the 21–40 (45%) and 41–60 (39%) years age groups when compared to the >60 years age group (28%; fig. 1b). While

Table 3. Percentage of HHcys in borderline B12 deficiency subjects

Parameter	21–40 years age group (n = 89)	41–60 years age group (n = 64)	>60 years age group (n = 73)	Pooled (n = 226)
Borderline deficiency of B12	37.1[a]	35.0[a]	35.4[a]	35.9
HHcys	80.9[a]	67.8[a]	79.2[a]	75.3

Pooled data represent the total number of borderline B12 deficiency samples (n = 226). Data represented in %, and significant differences ($p < 0.05$) among the age groups are indicated by superscript letters (a).

the overall prevalence of B12 deficiency was significantly higher in men (44%) than in women (29%; fig. 1b), FA deficiency was comparable between the genders (fig. 1a). After adjusting the BMI, Hb and lipid profile, the relationship between B12 and FA with age was unchanged. Irrespective of age and gender, as envisaged, the B12 deficiency was higher in the vegetarians (54%) than in those subjects consuming the mixed diet (31%) and the reverse was the case with FA, where the deficiency was higher in the mixed diet group (12%) than in the vegetarians (3%). Even amongst the vegetarians and mixed diet group, the B12 deficiency was higher in men than in women (70 vs. 43% and 38 vs. 24%) despite the higher dietary intake in men.

However, it should be noted that the concentration of B12 between 203 and 350 pg/ml [37, 38] accompanied by elevated plasma Hcys concentration (>12 μmol/l) was considered as borderline deficiency of B12. Therefore, we have also analyzed the concentration of Hcys in the samples to confirm borderline deficiency of B12. While the overall prevalence of borderline B12 deficiency was found to be 36%, there is no significant difference among the 3 age groups (table 3). The mean plasma Hcys concentration in the borderline B12 deficiency subjects is shown in table 1, and as expected, the prevalence of HHcys (>12 μmol/l) was about 75% in these subjects (table 3). Furthermore, we examined the correlation of Hcys with the corresponding B12 and FA levels. There was an inverse relationship between B12 ($r = 0.199$, $p = 0.00$) and FA ($r = 0.304$, $p = 0.00$) with Hcys.

There was no significant difference in the median dietary intake of FA and B12 among the age groups as assessed by a 3-day 24-hour dietary recall method (table 2). However, 66% of the study population with respect to FA and only 40% of the study population with respect to B12 was meeting 70% of the RDA (table 4). The dietary intake of FA with respect to vegetarians and mixed diet was significantly different in the 21–40 and 41–60 years age groups, whereas no significant difference was observed in the >60 years age group and also in the pooled group (fig. 2a). In the case of B12, the dietary intake with respect to the vegetarian and mixed diet group was significantly different in the 41–60 years age group and the pooled group but a trend was observed in the 21–40 years age group (fig. 2b). While no significant difference was found in dietary intake of FA between the genders in both the vegetarian and mixed diet group (fig. 3a), a significant difference in dietary intake of B12 was seen in men with the vegetarian and mixed diet group (fig. 3b).

Table 4. Percentages of nutrient adequacies of FA and B12 (≥70 RDA)

Age groups	FA	B12
21–40 years	58.8[a]	52.6[a]
41–60 years	65.7[a]	31.6[b]
>60 years	68.8[a]	33.8[b]
Pooled	65.6	39.7
χ^2	2.5	10.6
p value	0.285	0.05

Values represent percentages. Significant differences ($p < 0.05$) of values among the age groups are indicated by different superscript letters (a, b).

The relationship between the plasma status and the dietary intake of FA and B12 is depicted in table 5. While a significant correlation was found between the plasma status and the dietary intake of B12, the plasma status and the dietary intake of FA were not correlated. There was an association between the plasma B12 and the plasma FA. Furthermore, there was also a significant correlation between the dietary FA and the dietary B12. Correlation between B12 and FA with respect to the demographic and other biochemical parameters is shown in table 6. BMI and Hb showed a significant but inverse correlation with the B12 ($p < 0.01$). FBS was significantly associated with

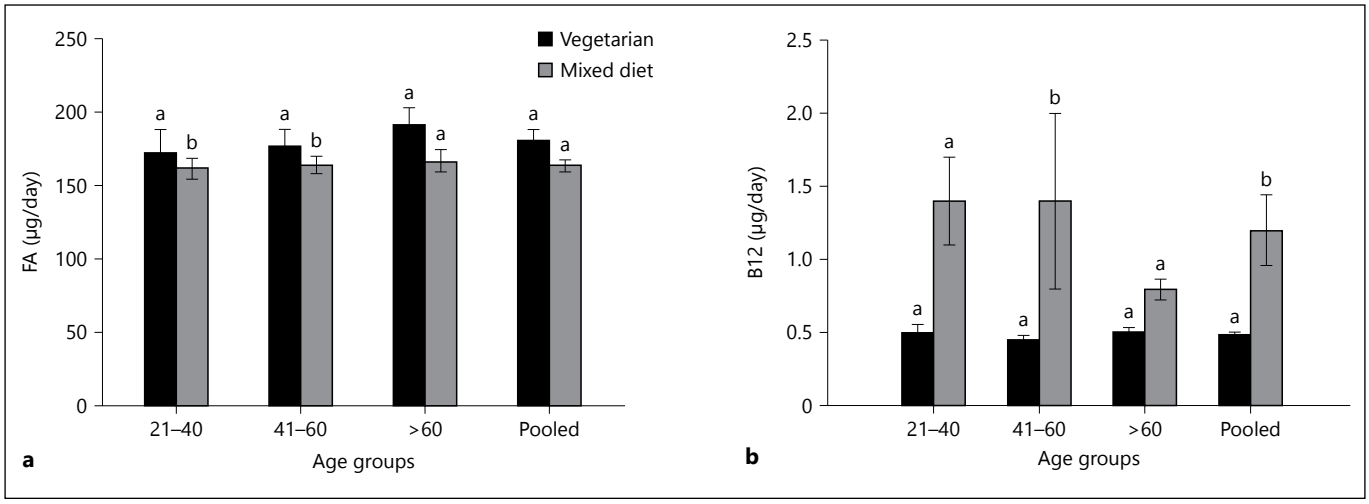

Fig. 2. Dietary intakes of FA (**a**) and B12 (**b**) among the age groups with different food habits. Data represent mean ± SE. Mean values across the age groups were compared by one-way ANOVA 'F' test with least significant difference. Significant differences ($p < 0.05$) of mean values among the age groups are indicated by letters (a, b) above the bars.

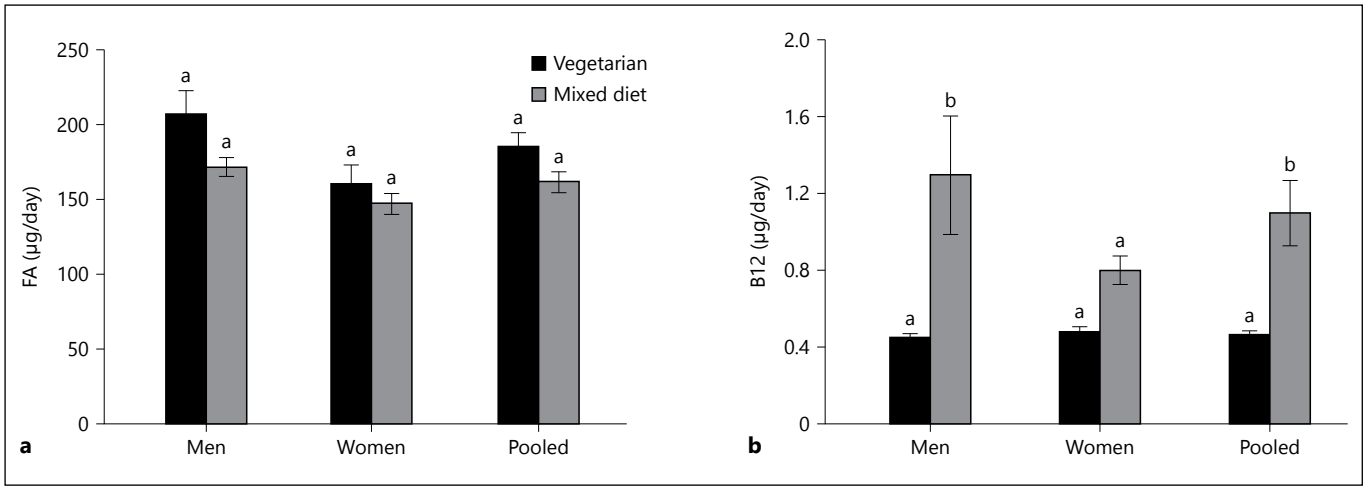

Fig. 3. Dietary intakes of FA (**a**) and B12 (**b**) between the genders with different food habits. Data represent mean ± SE. Mean values across age groups were compared by one-way ANOVA 'F' test with least significant difference. Significant differences ($p < 0.05$) of mean values among the age groups are indicated by letters (a, b) above the bars.

the B12. Hb correlated with plasma FA concentrations. A significant correlation was found between the plasma B12 and FA.

Discussion

Evidence suggests a massive burden of micronutrient deficiency in South Asia. Among the micronutrients, B12 and FA are of particular interest due to their diverse biological functions and roles in various chronic diseases [1]. This study was conducted in a South Indian urban setup on apparently healthy individuals of 21–85 years. The results of this study reveal that the prevalence of B12 deficiency is a leading nutritional problem of public health concern among the urban adult population. The observations identify some interesting points: the deficiency of B12 is more prevalent than FA, the overall prevalence of B12 deficiency (35%) is striking and it is more prevalent in the younger age groups than in the elderly as against

Table 5. Relationship between plasma status and dietary intake of FA and B12

Parameter	Plasma FA		Plasma B12		Dietary FA		Dietary B12	
	r value	p value	r value	p value	r value	p value	r value	p value
Plasma FA	–	–	0.126	0.015	0.051	0.324	–0.057	0.270
Plasma B12	0.126	0.015	–	–	–0.095	0.068	0.118	0.022
Dietary FA	0.051	0.324	–0.095	0.068	–	–	0.152	0.003
Dietary B12	–0.057	0.270	0.118	0.022	0.152	0.003	–	–

Correlations (r value) were assessed by the Spearman rank correlation. While positive r value indicates direct correlation, negative r value indicates an inverse relationship between the variables. Values of $p < 0.05$ were considered significant.

Table 6. Correlations of B12 and FA with demographic and biochemical parameters

Parameter	Plasma B12		Plasma FA	
	r value	p value	r value	p value
Age	–0.002	0.947	–0.05	0.130
BMI	–0.148	0.000	–0.034	0.334
Hb	–0.148	0.000	0.080	0.017
FBS	0.099	0.007	–0.43	0.242
HbA1c	0.032	0.371	–0.20	0.572
B12	–	–	0.179	0.000
FA	0.179	0.000	–	–

Correlations (r value) were assessed by the Spearman rank correlation. While positive r value indicates direct correlation, negative r value indicates an inverse relationship between the variables. Values of $p < 0.05$ were considered significant.

some reported studies [39, 40]. Furthermore, a significant (75%) proportion of subjects with borderline B12 deficiency are found to have HHcys. Shobha et al. [18] reported 16% prevalence of B12 deficiency in South Indian urban elderly population. Though the socioeconomic background of the present study population is somewhat similar to that in the study of Shobha et al. [18], the reported low level of deficiency was attributed to a high percentage of the study population consuming multivitamins and also the fact that the cohort belongs to a slightly higher income group. In the present study, in the case of FA, though the overall deficiency is 12%, it was more prevalent in the elderly (18%) compared to the younger age groups. Interestingly, the prevalence of B12 deficiency is significantly higher in men (44%) than in women (29%). As expected, B12 deficiency is higher in vegetarians (54%) than those on the mixed-diet (31%), but the reverse was the case with FA.

A few other studies support the findings of the present study that the deficiency of B12 is more prevalent than FA. Low B12 concentrations are common in Indians and contribute to HHcys despite normal FA status [21, 39]. A study in North Indian urban slum and non-slum areas reported markedly decreased dietary intakes of FA and B12, and conspicuous HHcys is implying an increased risk of cardiovascular diseases in Indians [28]. However, they did not determine blood concentrations of B12 and FA. A study from Pune also reported similar observations about 75% of the urban population having metabolic evidence (HHcys and methylmalonic acidemia) consistent with the cobalamin deficiency in Asian Indians [39]. The prevalence of HHcys among Asian Indian men residing in the United Kingdom was shown to be associated with cobalamin deficiency, and it was attributed to vegetarian diets [41]. Furthermore, it should be noted that the balance between B12 and FA is important than their individual status alone [42], and this study highlights this balance.

A comprehensive review of the literature showed a relatively significant deficiency prevalence of B12 among the vegetarians [43]. Furthermore, low B12 concentration and HHcys are common in Indian men, the majority being the vegetarians and middle-class urban residents [21, 44]. Although, more than 50% of our subjects are on mixed diet and the B12 intake is significantly higher in the mixed diet group compared to the vegetarians, the animal protein in their diet mainly came from the milk, occasional eggs and very rarely meat. Most of the subjects consumed wheat, rice, pulses, vegetables and ~250 ml of milk per day. Further investigations are warranted to understand the underlying factors for the B12 deficiency. Micronutrients are known to be associated with various chronic diseases such as type 2 diabetes and its complications, obesity and cancer. Recently, we reported altera-

tions in some micronutrients, particularly B12, vitamin D and some minerals in type 2 diabetes and its complications [30, 45, 46]. The findings of the present study further substantiate the general prevalence of B12 deficiency and are also in tune with our recent observations that about 40% adults above 50 years are B12 deficient [30].

In conclusion, the findings of this study show a higher prevalence of B12 deficiency in the apparently healthy individuals in an urban setup across the age groups. While the results are in concurrence with the studies that reported B12 status in different groups and under various conditions, at the same time, the findings indicate that factors other than dietary intake influence B12 deficiency. For example, the prevalence of deficiency in the younger adults is more than that in the elderly irrespective of similar dietary intake. Similarly, the prevalence of B12 deficiency was significantly higher in men than in women. Even amongst the vegetarian and mixed diet groups, the B12 deficiency was higher in men than that in women despite the higher dietary intake in men. Most importantly, only 40% of the study population was meeting 70% of the RDA of B12. Though further studies are required to confirm or dispute these observations, this study highlights the need for routine screening for B12 status in the apparently normal adults and the need to evolve sustainable strategies to ensure adequate B12 intake so as to prevent the chronic complications associated with B12 deficiency.

Strengths and Limitations

The study was conducted among different adult age groups with adequate sample size. Blood status of B12 and FA was complemented with dietary intake data. However, lack of data on methylmalonic acid (MMA), a sensitive indicator of B12 deficiency, is a limitation of the study. Estimation of MMA would have given a better representation of B12 status.

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Disclosure Statement

None of the authors have any conflict of interest.

Authors' Contribution

G.B.R. designed the research. M.S.-1, T.S., M.S.-2, P.L., P.S., N.A., B.P.R. and M.S.R. conducted the research. G.B.R. provided essential reagents and materials. G.B.R., N.B., M.S.-1 and T.S. analyzed the data and performed statistical analysis. G.B.R., M.S.-1 and T.S. wrote the paper. G.B.R. took primary responsibility for the final content. All authors read and approved the final manuscript.

Ethical Standard Disclosure

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Institutional Ethics Committee of the National Institute of Nutrition. Written informed consent was obtained from the all the subjects.

References

1 Stover PJ: Physiology of folate and vitamin B12 in health and disease. Nutr Rev 2004; 62:S3–S12; discussion S13.
2 Nath SD, Koutoubi S, Huffman FG: Folate and vitamin B12 status of a multiethnic adult population. J Natl Med Assoc 2006;98:67–72.
3 Oh R, Brown DL: Vitamin B12 deficiency. Am Fam Physician 2003;67:979–986.
4 Reynolds E: Vitamin B12, folic acid, and the nervous system. Lancet Neurol 2006;5:949–960.
5 Shane B, Stokstad EL: Vitamin B12-folate interrelationships. Annu Rev Nutr 1985;5:115–141.
6 Villamor E, Mora-Plazas M, Forero Y, et al: Vitamin B-12 status is associated with socioeconomic level and adherence to an animal food dietary pattern in Colombian school children. J Nutr 2008;138:1391–1398.
7 Ray JG, Cole DE, Boss SC: An Ontario-wide study of vitamin B12, serum folate, and red cell folate levels in relation to plasma homocysteine: is a preventable public health issue on the rise? Clin Biochem 2000;33:337–343.
8 Hinds HE, Johnson AA, Webb MC, Graham AP: Iron, folate, and vitamin B12 status in the elderly by gender and ethnicity. J Natl Med Assoc 2011;103:870–877.
9 Pflipsen MC, Oh RC, Saguil A, et al: The prevalence of vitamin B(12) deficiency in patients with type 2 diabetes: a cross-sectional study. J Am Board Fam Med 2009;22:528–534.
10 Quinlivan EP, McPartlin J, McNulty H, et al: Importance of both folic acid and vitamin B12 in reduction of risk of vascular disease. Lancet 2002;359:227–228.

11 Thompson MD, Cole DE, Ray JG: Vitamin B-12 and neural tube defects: the Canadian experience. Am J Clin Nutr 2009;89:697S–701S.
12 Medrano MJ, Sierra MJ, Almazan J, et al: The association of dietary folate, B6, and B12 with cardiovascular mortality in Spain: an ecological analysis. Am J Public Health 2000;90: 1636–1638.
13 He K, Merchant A, Rimm EB, et al: Folate, vitamin B6, and B12 intakes in relation to risk of stroke among men. Stroke 2004;35:169–174.
14 Alberg AJ, Selhub J, Shah KV, et al: The risk of cervical cancer in relation to serum concentrations of folate, vitamin B12, and homocysteine. Cancer Epidemiol Biomarkers Prev 2000;9:761–764.
15 Glynn SA, Albanes D, Pietinen P, et al: Colorectal cancer and folate status: a nested case-control study among male smokers. Cancer Epidemiol Biomarkers Prev 1996;5: 487–494.
16 Ibiebele TI, Hughes MC, Pandeya N, et al: High intake of folate from food sources is associated with reduced risk of esophageal cancer in an Australian population. J Nutr 2011; 141:274–283.
17 Christen WG, Glynn RJ, Chew EY, et al: Folic acid, pyridoxine, and cyanocobalamin combination treatment and age-related macular degeneration in women: the Women's Antioxidant and Folic Acid Cardiovascular Study. Arch Intern Med 2009;169:335–341.
18 Shobha V, Tarey SD, Singh RG, et al: Vitamin B12 deficiency & levels of metabolites in an apparently normal urban south Indian elderly population. Indian J Med Res 2011;134:432–439.
19 Taneja S, Bhandari N, Strand TA, et al: Cobalamin and folate status in infants and young children in a low-to-middle income community in India. Am J Clin Nutr 2007;86:1302–1309.
20 Bhardwaj A, Kumar D, Raina SK, et al: Rapid assessment for coexistence of vitamin B12 and Iron deficiency anemia among adolescent males and females in Northern Himalayan state of India. Anemia 2013;2013:959605.
21 Yajnik CS, Deshpande SS, Lubree HG, et al: Vitamin B12 deficiency and hyperhomocysteinemia in rural and urban Indians. J Assoc Physicians India 2006;54:775–782.
22 Agarwal R: Vitamin B12 deficiency & cognitive impairment in elderly population. Indian J Med Res 2011;134:410–412.
23 Veena SR, Krishnaveni GV, Srinivasan K, et al: Higher maternal plasma folate but not vitamin B-12 concentrations during pregnancy are associated with better cognitive function scores in 9- to 10- year-old children in South India. J Nutr 2010;140:1014–1022.
24 Chandra J, Jain V, Narayan S, et al: Folate and cobalamin deficiency in megaloblastic anemia in children. Indian Pediatr 2002;39:453–457.
25 Sukla KK, Tiwari PK, Kumar A, Raman R: Low birthweight (LBW) and neonatal hyperbilirubinemia (NNH) in an Indian cohort: association of homocysteine, its metabolic pathway genes and micronutrients as risk factors. PLoS One 2013;8:e71587.
26 Yajnik CS, Deshpande SS, Jackson AA, et al: Vitamin B12 and folate concentrations during pregnancy and insulin resistance in the offspring: the Pune Maternal Nutrition Study. Diabetologia 2008;51:29–38.
27 Krishnaveni GV, Veena SR, Karat SC, et al: Association between maternal folate concentrations during pregnancy and insulin resistance in Indian children. Diabetologia 2014; 57:110–121.
28 Misra A, Vikram NK, Pandey RM, et al: Hyperhomocysteinemia, and low intakes of folic acid and vitamin B12 in urban North India. Eur J Nutr 2002;41:68–77.
29 Abraham R, John MJ, Calton R, Dhanoa J: Raised serum homocysteine levels in patients of coronary artery disease and the effect of vitamin B12 and folate on its concentration. Indian J Clin Biochem 2006;21:95–100.
30 Satyanarayana A, Balakrishna N, Pitla S, et al: Status of B-vitamins and homocysteine in diabetic retinopathy: association with vitamin-B12 deficiency and hyperhomocysteinemia. PLoS One 2011;6:e26747.
31 Gulvady C, Pingle S, Shanbhag S: Incidence of vitamin B12/D3 deficiency among company executives. Indian J Occup Environ Med 2007;11:83–85.
32 de Benoist B: Conclusions of a WHO Technical Consultation on folate and vitamin B12 deficiencies. Food Nutr Bull 2008;29:S238–S244.
33 Gopalan C RB, Balasubramanyam SC, Narsinga Rao BS, Deosthale TG & Pant KC: Nutritive Value of Indian Foods. National Institute of Nutrition, Hyderabad, 1990.
34 ICMR: Nutrient Requirements and Recommended Dietary Allowances for Indians. Expert Group of Indian Council of Medical Research, New Delhi, 2010.
35 NNMB: Report on food and nutrient intake of individuals: National Nutrition Monitoring Bureau Special Report. National Institute of Nutrition, 2000.
36 USDA: Composition of Foods: Raw, Processed, Prepared. USDA National Nutrient Database for Standard Reference. Betsville, 2011.
37 El-Khateeb M, Khader Y, Batieha A, et al: Vitamin B12 deficiency in Jordan: a population-based study. Ann Nutr Metab 2014;64:101–105.
38 Clarke R, Refsum H, Birks J, et al: Screening for vitamin B-12 and folate deficiency in older persons. Am J Clin Nutr 2003;77:1241–1247.
39 Refsum H, Yajnik CS, Gadkari M, et al; Hyperhomocysteinemia and elevated methylmalonic acid indicate a high prevalence of cobalamin deficiency in Asian Indians. Am J Clin Nutr 2001;74:233–241.
40 Magnus EM, Bache-Wiig JE, Aanderson TR, Melbostad E: Folate and vitamin B12 (cobalamin) blood levels in elderly persons in geriatric homes. Scand J Haematol 1982;28:360–366.
41 Chambers JC, Obeid OA, Refsum H, et al: Plasma homocysteine concentrations and risk of coronary heart disease in UK Indian Asian and European men. Lancet 2000;355: 523–527.
42 Yajnik CS, Deshmukh US: Fetal programming: maternal nutrition and role of one-carbon metabolism. Rev Endocr Metab Disord 2012;13:121–127.
43 Pawlak R, Lester SE, Babatunde T: The prevalence of cobalamin deficiency among vegetarians assessed by serum vitamin B12: a review of literature. Eur J Clin Nutr 2014;68:541–548.
44 Pitla S, Nagalla B: Gender-related differences in the relationship between plasma homocysteine, anthropometric and conventional biochemical coronary heart disease risk factors in middle-aged Indians. Ann Nutr Metab 2009; 54:1–6.
45 Reddy GB, Sivaprasad M, Shalini T, et al: Plasma vitamin D status in patients with type 2 diabetes with and without retinopathy. Nutrition 2015;31:956–963.
46 Reddy GB, Satyanarayana A, Balakrishna N, et al: Micronutrient status in diabetic retinopathy. Annals Nutr Metab 2013;63:1455.