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Vitamin B_{12} deficiency & levels of metabolites in an apparently normal urban south Indian elderly population

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***Background & objectives*: There is no published literature on the extent of vitamin B_{12} deficiency in elderly Indians as determined by plasma vitamin B_{12} levels and methylmalonic acid (MMA) levels. Vitamin B_{12} deficiency is expected to be higher in elderly Indians due to vegetarianism, varied socio-economic strata and high prevalence of *Helicobacter pylori* infection. We therefore, studied the dietary habits of south Indian urban elderly population and measured vitamin B_{12}, MMA red cell folate and homocysteine (Hcy) levels.**

***Methods*: Healthy elderly urban subjects (175, >60 yr) were recruited. Detailed history, physical examination and neurological assessment were carried out. Food Frequency Questionnaire (FFQ) for dietary analysis for daily intake of calories, vitamin B_{12}, folate and detailed psychological assessment for cognitive functions was carried out. Blood samples were analyzed for routine haematology and biochemistry, vitamin B_{12}, red cell folate, MMA and Hcy.**

***Results*: The mean age of the study population was 66.3 yr. Median values for daily dietary intake of vitamin B_{12} and folate were 2.4 and 349.2 µg/day respectively. Sixty two (35%) participants consumed multivitamin supplements. Plasma vitamin B_{12} level and the dietary intake of vitamin B_{12} was significantly correlated (*P*=0.157). Plasma vitamin B_{12} and Hcy were inversely correlated (*P*= -0.509). Red cell folate was inversely correlated with Hcy (*P*= -0.550). Significant negative correlation was observed between plasma vitamin B_{12} and MMA in the entire study population (*P*= -0.220). Subjects consuming vitamin supplements (n=62) had significantly higher plasma vitamin B_{12} levels, lower MMA levels and lower Hcy levels. There was no significant correlation between plasma vitamin B_{12}, MMA, Hcy and red cell folate and any of the 10 cognitive tests including Hindi Mental Status Examination (HMSE).**

***Interpretation & conclusions*: Our study is indicative of higher vitamin B_{12} (2.4 µg/day) intakes in urban south Indian population. Thirty five per cent of the study population consumed multivitamin supplements and therefore, low plasma vitamin B_{12} levels were seen only in 16 per cent of the study subjects. However, MMA was elevated in 55 per cent and Hcy in 13 per cent of the subjects.**

Key words Cognitive assessment - geriatric - methylmalonic acid - nutrition - vitamin B_{12} deficiency

Over the last few decades, many investigators have reported a high prevalence of vitamin B_{12} deficiency in elderly population[1-3]; although a small minority has disputed this incidence[4-6]. The published prevalence of subnormal vitamin B_{12} concentration in elderly varies from 3-40.5 per cent depending on the cut-off used for defining deficiency of cobalmine level in serum[1]. The unique value of blood methylmalonic acid (MMA) as a specific marker of functional vitamin B_{12} is also established. It has been demonstrated that elderly population has higher MMA levels which exponentially increases with age. Blood MMA has proved to be a reliable indicator of functional vitamin B_{12} status rather than serum vitamin B_{12} levels in elderly population[3,7,8].

There is no information on the extent of vitamin B_{12} deficiency in elderly Indians as determined by plasma vitamin B_{12} levels. More importantly, there is no data on functional vitamin B_{12} status in elderly Indians as determined by blood MMA levels. We hypothesize that vitamin B_{12} deficiency may be higher in elderly Indians due to vegetarianism, varied socio-economic strata and high prevalence of *Helicobacter pylori* infection[9]. We undertook this study to collect data on the dietary habits of urban south Indian elderly subjects, measure their plasma vitamin B_{12} levels, MMA levels, red cell folate and homocysteine (Hcy) levels. In addition, as deficiency of vitamin B_{12} and elevated Hcy levels have been shown to reduce cognitive performance in elderly[10,11], cognitive functions in this urban south Indian elderly population were also studied.

Material & Methods

Subjects: One hundred and seventy five healthy men and women (>60 yr) were recruited between April and December 2009 from various Senior Citizen Associations in Bangalore (84%) and outpatient department at St. John's Medical College Hospital (14%), Bangalore. The subjects were recruited based on convenience sampling technique. Sample size was estimated based on expected prevalence of vitamin B_{12} deficiency of 30 per cent with a relative precision of 25 per cent. This was estimated to be 150. The purpose of the study was explained and a written consent was obtained from each of the participants. Demographic, socio-economic and lifestyle information was obtained using a questionnaire. Education was recorded as completed years of formal education and monthly income was categorized.

Detailed history, physical examination and neurological assessment were carried out by a qualified physician. Medical history that included gastrointestinal (GI) symptoms, neurologic and psychiatric symptoms, underlying medical and surgical conditions, was obtained using a structured questionnaire. Subjects with underlying heart disease, renal failure, stroke and major surgery within the last year were excluded. Subjects with previous neurologic or psychiatric symptoms were also excluded. This ensured inclusion of only healthy elderly subjects in this study. Subjects with well controlled diabetes and hypertension were included. The Institutional Ethical Review Board of St. John's Medical College approved the research protocol.

Nutritional assessment: Habitual dietary intake for the preceding six months was assessed using a food frequency questionnaire[12] that was interviewer administered by a trained nutritionist. This questionnaire was adapted from the one developed for the urban middle class residing in south India. It has a food list of 108 items, derived from a food database developed over a period of many years from studies at the Division of Nutrition, St John's Medical College, and has four frequency categories (daily, weekly, monthly and yearly)[12]. Nutrient composition of the food item was calculated using standard food conversion tables for the ingredients[12]. Wherever available, Indian data were used. However, for some nutrients, for which Indian data were not available, USDA data in the public domain were used[13]. A replica sheet of the questionnaire was made in Microsoft Excel and the information was entered. The program computed nutrient scores by multiplying the relative frequency of consumption of each food item by its nutrient content of the standard portion size. Nutrient information was obtained on total caloric intake, daily folate and vitamin B_{12} intake and many other macro- and micro-nutrients. Information on dietary supplements being consumed was separately recorded to arrive at the total daily intake of vitamin B_{12}.

Neuropsychological assessment: The cognitive measures consisted of a series of neuropsychological tests applicable for use in elderly population. The cognitive battery included neuropsychological tests specially adapted from the Consortium to Establish a Registry for Alzheimer's disease (CERAD)[14] and the Indo-U.S. Cross National Dementia Epidemiology Study[15]. This battery consisted of 10 sub-tests, which measured various domains of cognitive abilities and was administered by a trained clinical psychologist. The cognitive domains that were assessed included verbal reproduction and language function, language capacity and impairment of expressive language or speech, mathematical abilities, attention and concentration, visual perception and motor execution and various aspects of memory such as immediate memory, delayed recall and delayed recognition. In addition, subjects were also assessed on an Indian adaptation of "Mini Mental Status Examination" (MMSE) - a widely used screening instrument for the detection of cognitive deficits[16]. The Indian adaptation of the "Mini Mental Status Examination" is known as the "Hindi Mental Status Examination" (HMSE)[17]. The Kannada version of this instrument was used to suit the local population; this was done using the translation-back translation procedure[18]. Depression, which can confound assessment of cognitive abilities in elderly, was measured on "Geriatric Depression Scale"[19]. Finally, the functional status of the subjects was assessed using "everyday ability scale"[20] adapted for Indian population.

Biochemical measurements: Approx. 4 ml of blood was collected from each subject and routine haematological work up, including haemoglobin, total leucocyte count, platelet count, haematocrit, red cell indices, blood smear evaluation, neutrophil lobe count, blood sugars and serum creatinine was done on all the subjects using standard haematology and biochemical techniques. Whole blood was treated with ascorbic acid and stored for red cell folate analysis. The plasma was separated and stored at -80°C until analysis for vitamin B_{12}, Hcy and MMA. Red cell folate and vitamin B12 was measured by the electrochemiluminescence method (Elecsys 2010, Roche Diagnostics Mannheim, USA). The intra- assay coefficient of variation (CV) for the trilevel level controls for red cell folate and vitamin B_{12} were 2.6, 2.4, 1.7; 4.6, 4.2, 2.8; and inter-assay were 6.4,5.1,5.8; 6.8, 5.3, 3.2; respectively.

The combined measurement of Hcy and MMA was performed by gas chromatography-mass spectrometry (GC-MS) method. An aliquote of 500 µl of plasma was lyophilized in a freeze dryer (Labconco, Kansas, MO, USA) to concentrate the analytes. The lyophilized mass was reconstituted with 200 µl of MiliQ water. The plasma was treated with D,L-dithioerythritol containing deuterated homocysteine (d_8-Hcy), to cleave disulphide bonds in the deuterated homocysteine, protein and cysteine bound homocysteine and dimeric form of homocysteine. Plasma was deproteinized by ethanol containing deuterated methyl malonic acid (d_3-MMA). The supernatant containing Hcy, MMA and their deuterated standards were derivatized with methycholorformate and extracted into toluene. The N(S)-methoxycarbonyl ethyl ester derivatives in the extract were injected onto the GC-MS (Varian 3800, Palo Alto, CA, USA) and separated on a CP sil 24-CB low-bleed/MS capillary column (15m X 0.25 mm (i.d); film thickness, 0.25 µm) from Varian, USA. The molecules were analyzed in the selective ion monitoring mode of the MS. The ion pairs of *m/z* 174/177 for MMA/d_3-MMA and 233/237 for Hcy/d_4-Hcy were quantified. The concentrations of Hcy and MMA were computed from calibration curves drawn using area ratios of analyte and deuterated analyte, against known concentration of the analyte. The intra- and inter-assay CV for Hcy and MMA were 4.6, 6.2 and 9.3, 10.1 respectively[21].

Statistical analysis: Data recorded on a pre-designed proforma were entered on an excel spreadsheet and entries double checked for any errors. Normality was checked using Kolmogorov-Smirnov (KS) test. Vitamin B_{12} intake and plasma levels of MMA and Vitamin B_{12} were not normally distributed, and therefore, non-parametric statistical tests were used for vitamin B_{12} and its metabolites. Categorical data were presented using number and percentage and continuous data as median and quartiles. Since plasma vitamin B_{12} levels were not normally distributed, Spearman's correlation was performed between vitamin B_{12} and the metabolites to assess the extent of their correlation. The data were divided into two groups based on supplement intakes and were compared using Mann-Whitney U test. Categorical variables were compared using Chi-square test. The cognitive parameters were compared between the study groups using Mann-Whitney U test. $P<0.05$ was considered significant. Data were analyzed using SPSS version 11.5.

Results

Anthropometric and demographic characteristics: One hundred and seventy five elderly subjects voluntarily participated in this study. The mean BMI was 24.4 ± 3.8 kg/m2 and the mean weight and height were 63.1 ± 10.2 kg and 161.1 ± 9.4 cm, respectively. The mean age of the study population was 66.3± 6.8 yr and 22 per cent (n=38) of the participants were older than 70 yr. The number of men and women were almost equal and 68 per cent of subjects reported an educational qualification of graduation and above. All the subjects belonged to urban areas of Bangalore city and 75 per cent (n=131) belonged to upper middle socio-economic

Table I. Demographic characteristics of the subjects

	No. (%)
Gender	
Males	91 (52.0)
Females	84 (48.0)
Socio-economic status	
Upper	8 (4.6)
Upper middle	131 (74.8)
Lower middle	24 (13.7)
Upper lower	11 (6.3)
Lower	1 (0.5)
Food habits (n=174)	
Vegetarians	99 (57.0)
Non vegetarians	75 (43.0)
Chronic ailments	
Diabetes	15 (8.5)
Hypertension	48 (27.4)
Both diabetes and hypertension	17 (9.7)
Education	
Post graduation	45 (25.7)
Graduation	74 (42.3)
Diploma/post high school	23 (13.1)
High school & below	15 (8.8)

n=175, except where specified

strata[19]. Three-fourth of the subjects were walking at least once a week for 30 min or more. All were healthy on physical examination. Well-controlled diabetes and/or hypertension was encountered in 45.6 per cent of the subjects (Table I).

Nutrient consumption and blood levels of nutrients and metabolites: Vegetarianism was observed in 57 per cent (n=99) of the study population. The range and median (lower, upper quartiles) of daily dietary intakes of vitamin B_{12} and folate and blood levels of the vitamins and their metabolites are described in Table II. The recommended daily intake of vitamin B_{12} and folate is 2 µg/day and 400 µg/day respectively[21,22]. Daily vitamin B_{12} consumption was lesser than recommended daily intake in 25 per cent of the subjects; of these 31 per cent were vegetarians. Fifty one per cent of the study population was consuming less than recommended daily intake of folate. The average (mean ± SD) daily caloric intake of the given population was 1883±467 cals, carbohydrate was 277±68 g, protein was 56±14 g and fat was 61±19 g.

Median haemoglobin (Hb) of the population was 13.4 g/dl (Table II) and low Hb levels were noted in 14 per cent (n=25) of the subjects, two third of whom were men. There was no significant correlation between Hb, mean corpuscular volume (MCV) and plasma vitamin B_{12} levels. All the subjects had normal serum creatinine levels. Plasma levels of vitamin B_{12} were subnormal (< 150 pmol/l) in 16 per cent of the study population whereas, elevated MMA (>0.3 µmol/l) was found in 55 per cent of the population. Elevated Hcy levels were found in 13 per cent of the subjects and red cell folate was normal for all the subjects (Table III). Women (90%) were found to have normal vitamin B_{12} levels (≥150 pmol/l) as compared with men (78%) (P=0.025), similarly most women (96%) had normal Hcy levels (≤15 µmol/l) compared with men (79%) (P=0.001).

Plasma vitamin B_{12} level and the dietary intake of vitamin B_{12} were not significantly correlated. Significant negative correlation was observed between plasma vitamin B_{12} and MMA for the entire data set (ρr= -0.22). Plasma vitamin B_{12} and red cell folate were inversely correlated (ρ= -0.509, ρ= -0.550 respectively) with Hcy. Age had no significant correlation with plasma vitamin B_{12} levels (ρ= -0.03), MMA levels (ρ= -0.05) or Hcy levels (ρ=0.14) (Table IV). Vitamin B_{12} was divided into quintiles of its distribution and scatter plot of the median for each quintile, for vitamin B_{12} and MMA and; vitamin B_{12} and Hcy was plotted (Fig. a & b).

Table II. Intakes of micronutrients, biochemical status and haematological indices in study subjects

	Median (Q1,Q3)	Min-Max
Folate dietary intake (µg/day) (n=174)	349.2 (294.8,425.9)	160.4 - 745.5
Vitamin B_{12} dietary intake (µg/day) (n=174)	2.4 (1.7,3.2)	0.5 - 11.9
Red Cell folate (ng/ml) (n=156)	1045.4 (778.7,1316.9)	508.2 – 2958.3
Plasma vitamin B_{12} (pmol/l)	306.9 (180.9,598.8)	49.1 - 1476.0
Plasma methylmalonic acid (µmol/l)	0.33 (0.21,0.58)	0.01 - 3.58
Plasma homocysteine (µmol/l)	9.3 (7.2,11.6)	2.0 - 28.4
Serum creatinine (mg/dl) (n=172)	0.8 (0.6,0.9)	0.2 – 1.3
Haemoglobin (g %) (n=174)	13.4 (12.5,14.4)	8.3 - 16.4
Mean corpuscular volume (fl) (n=173)	86.4 (83.9,89.6)	54.3 - 98.3

n = 175, except where specified. (Q1,Q3) = (lower quartile, upper quartile)

Table III. Deficiency of micronutrients based on intake and biochemical status

		Vitamin B_{12} intake (<2 µg/day) %	Folate intake (<400 µg/day) %	Vitamin B_{12} (<150 pmol/l) %	MMA (>0.3 µmol/l) %	Homocysteine (>15 µmol/l) %
Whole group		25.0	51.0	16	54.9	12.6
Age (yr)	>70 (n=38)	28.9	44.7	7.9	52.6	15.8
	≤70 (n=136)	24.2	52.9	18.2	55.5	11.7
Gender	Men (n=91)	23.0	48.3	22.0	61.5	20.9
	Women (n=84)	27.7	54.2	9.5	47.6	3.6
Dietary preferences	Vegetarians (n=99)	31.3	56.5	19.1	61.6	17.1
	Non vegetarians (n=76)	17.1	43.4	11.8	46	6.5

n = 175, except as indicated in Table II. MMA, methylmalonic acid

Table IV. Bivariate correlations of nutrient intakes and metabolites

	Age	Vitamin B_{12}	MMA	Red cell folate	Homocysteine	Folate intake	Vitamin B_{12} intake
Age	1.0						
Vitamin B_{12}	-0.03	1.0					
MMA	-0.05	-0.22**	1.0				
Red cell folate	0.11	0.48**	-0.13	1.0			
Homocysteine	0.14	-0.51**	0.23**	-0.55**	1.0		
Folate dietary intake	-0.21**	-0.10	-0.03	-0.02***	-0.08	1.0	
Vitamin B_{12} dietary Intake	-0.11	0.16*	-0.19	-0.00	-0.04	0.174*	1.0

n = 175, except as indicated in Table II. MMA, methylmalonic acid. P ***<0.001, **<0.01, *<0.05.

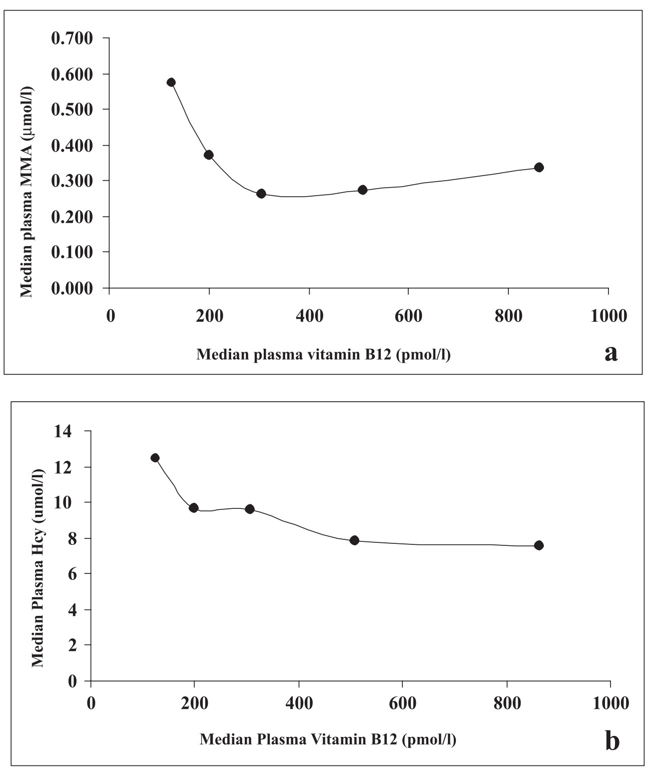

Fig. Scatter plot of the median for each quintile: **(a)** for vitamin B_{12} and MMA and **(b)** for vitamin B_{12} and Hcy.

Analysis of supplemental vitamin intake: Multivitamin supplements were consumed almost on a daily basis by 35 per cent (n=61) of the participants and, therefore, subjects were categorized as supplement consumers and non-consumers. The range and median (lower, upper quartiles) of the dietary intakes and plasma concentration for the two groups are described in Table V. The median (lower, upper quartiles) and range of total intake (diet and supplements) of vitamin B_{12} and folate for the supplement consumers was 16.9 (10.2, 18.0), 3.31 - 33.64 µg/day and 1757 (501, 1862), 170 - 2141 µg/day respectively. Thirty one per cent of the subjects who were taking supplements had dietary deficiency of vitamin B_{12} as analyzed by food frequency questionnaire but only 5 per cent have low plasma vitamin B_{12} levels. In the supplement non-consumer group 39 per cent had dietary deficiency of vitamin B_{12} and 22 per cent had low plasma vitamin B_{12} levels.

Subjects consuming vitamin supplements (n=62) had significantly higher plasma vitamin B_{12}, lower MMA and Hcy levels compared with subjects who were not taking supplements (P<0.001). Only 2 subjects consuming supplements had higher than normal Hcy levels.

Table V. Analysis of supplemental vitamin intake

	Supplement consumers Median (Q1,Q3) Min-Max	Supplement non-consumers Median (Q1,Q3) Min-Max
Folate dietary intake (µg/day)	350.1 (298.2, 451.9) 170.6 - 641.2	349.2 (286.8, 415.2) 160.4 - 745.5
Vitamin B_{12} dietary intake (µg/day)	2.5 (1.8, 3.6) 0.6 - 11.9	2.2 (1.7, 3.1) 0.5 - 8.0
Red cell folate (ng/ml)	1217.2 (1047.1, 1637.0) 568.3 - 2958.3	878.4 (700.4, 111.6)*** 508.2 - 2321.2
Plasma vitamin B_{12} (pmol/l)	494.7 (300.9, 707.7) 118.6 - 1476.0	244.4 (152.2, 417.1)*** 49.1 - 1084.9
Plasma methylmalonic acid (µmol/l)	0.26 (0.17, 0.46) 0.01 - 1.43	0.37 (0.24, 0.66)*** 0.01 - 3.58
Plasma homocysteine (µmol/l)	7.6 (6.0, 9.0) 3.1 - 16.7	10.6 (8.4, 13.7)*** 2.0 - 28.4
Serum creatinine (mg/dl)	0.8 (0.7, 0.9) 0.2 - 1.3	0.8 (0.6, 0.9) 0.3 - 1.3
Haemoglobin (g%)	13.3 (12.4, 14.3) 11.1 - 16.0	13.4 (12.6, 14.6) 8.3 - 16.4
Mean corpuscular volume (fl)	88.0 (84.1, 90.6) 54.3 - 94.7	86.6 (83.7, 89.2) 57.4 - 98.3

n=62 for supplement consumers, except for red cell folate (n-58). n=113 for supplement non- consumers, except for red cell folate (n-98), serum creatinine (n=110), Mean corpuscular volume (n=111), haemoglobin, folate and vitamin B_{12} intakes (n=112). Mann Whitney T test between supplement consumers and non-consumers. ***P<0.001

Neuropsychological assessments: There was no significant correlation between plasma vitamin B_{12}, MMA, Hcy & red cell folate levels and any of the 10 cognitive tests. Age and gender did not influence any of the neuropsychological assessments. Subjects who had above high school education did better in Verbal Fluency for Categories (P=0.01), Naming Test (P=0.037), HMSE (P<0.001) and Calculation (P=0.011) in comparison to individuals with lower than high school education. In addition, Naming test score was significantly higher for those belonging to the upper and upper middle class socio-economic status (P=0.034). It was also observed that none of the subjects scored below the cut-off score of HMSE, which is 19[17].

Discussion

This study was carried out prospectively in an urban south Indian healthy elderly population. The findings of our study contradict the expected low plasma vitamin B_{12} levels and high MMA levels in elderly population because about 80 per cent of the sample belonged to either upper middle or upper income groups. There was a significant correlation between plasma vitamin B_{12} and MMA levels and a plot of the median vitamin B_{12} and MMA for each segment of quintiles of vitamin B_{12} distribution showed a nadir beyond 300 pmol/l of vitamin B_{12}. However, there was a non significant increase in plasma MMA concentration for subjects with vitamin B_{12} >400 pmol/l, and could be attributed to the non linear relationship of plasma vitamin B_{12} and MMA. A similar observation has been reported in a subset population of the British National Diet and Nutrition Survey[8]. Refsum *et al*[10] also observed an increasing serum MMA value from 0.84 µmol/l in young adults with vitamin B_{12} greater than 200 pmol/l to 2.64 µmol/l for vitamin B_{12} less than 100 pmol/l, and a plateau of the relationship between vitamin B_{12} and MMA was observed beyond a vitamin B_{12} concentration of 250 pmol/l[8].

Plasma levels of vitamin B_{12} were subnormal only in 16 per cent but elevated MMA was found in 55 per cent of the population. The elevated MMA in subjects with normal to high vitamin B_{12} can be explained by evaluating the determinants of MMA. Age and plasma creatinine contribute to only 17 per cent variability of MMA and data on other determinants are sparse[10,22]. Therefore, caution needs to be exercised in extrapolating MMA levels to indicate the prevalence of vitamin B_{12} deficiency.

Our study population was consuming a higher than recommended vitamin B_{12} in the daily diet. Almost half of the subjects were vegetarians; despite this the daily dietary intake of vitamin B_{12} was high. After adjusting for the vitamin supplements, the daily total intake of vitamin B_{12} was 3.5 times higher than the FDA recommendation[20]. It appeared that supplements had a significant impact on the normalization of the plasma vitamin B_{12} levels, MMA and Hcy levels. This is likely to have a significant effect on the elderly subjects' well-being and longevity. The average daily dietary intake of folate almost equals the FDA recommended daily intake of folate[22]. After considering the supplements, the total folate intake in our subjects was three times the recommended intake. This probably resulted in normal Hcy levels in the supplement-consuming group.

Refsum *et al*[10] have demonstrated vitamin B_{12} deficiency (<150 pmol/l) in 47 per cent and elevated MMA levels (>0.26 µmol/l) in 73 per cent of their adult (age 35-54 yr) subjects. However, only one fourth of them were healthy, while almost half had evidence of cardiovascular disease. Therefore, these results are not reflective of vitamin B_{12} deficiency in disease free adult Indian population. Yajnik *et al*[24] have attempted to assess prevalence of vitamin B_{12} deficiency in middle aged (33-46 yr) rural and urban men. Two third of them had low plasma vitamin B_{12} levels. In this study MMA levels were not analyzed. Both these studies from India have not used a validated and structured tool to assess dietary intake of vitamin B_{12}.

Surprisingly, the age related variations in plasma vitamin B_{12} levels and MMA were not observed in our study subjects in contrast to published literature[3,7,8]. This may be attributed to inclusion of only healthy subjects in a narrow age band. This study population was devoid of any cardiac, gastrointestinal, cerebrovascular diseases or psychiatric disorders. In addition, this population appeared to be very health conscious as indicated by their mean BMI, frequency of exercise and 'Off the counter' use of multivitamin supplements by one third of them. A few other investigators have also refuted age associated decline of vitamin B_{12} levels[4,6,25-27].

A lack of significant association between plasma vitamin B_{12} and MMA levels with cognitive measures could be due to a number of reasons. Firstly, low vitamin B_{12} levels were seen in only 16 per cent of study population. Secondly, the cognitive tests were chosen from a battery of neuropsychological tests that were standardized to detect dementia in a rural Indian population and may not be sensitive enough to detect subtle dysfunctions in cognitive abilities associated with nutritional deficiencies. Thirdly, excluding subjects who reported memory difficulties at the time of recruitment might have biased the sample. Finally, majority of the subjects were in the "young old" category with a few older than 70 yr of age.

This study contradicts the previous studies[10,27] which reported a high prevalence of clinical and subclinical vitamin B_{12} deficiency in urban middle class Indians. This also proves that contrary to perception, food vitamin B_{12} malabsorption may not be as common as was thought earlier. However, before any generalizations are made based on these observations about deficiency of vitamin B_{12} in elderly Indians especially vegetarians, studies on the prevalence of vitamin B_{12} deficiency in any subset of population should use a validated tool to quantify dietary intake of vitamin B_{12}. These observations, then, need to be corroborated with plasma vitamin B_{12}, Hcy and MMA levels. Additionally, normal values for MMA and vitamin B_{12} need to be established in Indian population. As per the 2001 census, 72.2 per cent of the Indian population[29] lives in about 6,38,000 villages[30] suggesting the need for similar studies on a larger scale in the rural and the underprivileged and other populations.

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