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ORIGINAL ARTICLE

Prevalence of Vitamin B_{12} and Folate Deficiency in School Children Residing at High Altitude Regions in India

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Abstract
Objective To assess the prevalence of vitamin B_{12} and folate deficiencies among children residing at high altitude regions of Himachal Pradesh, India.
Methods A total of 215 school children in the age group of 6–18 y were included. Biochemical estimation of serum vitamin B_{12} and folate levels was undertaken using chemiluminescence immunoassay method. The consumption pattern of foods high in dietary vitamin B_{12} and folate was recorded using Food Frequency Questionnaire.
Results The median levels (interquartile range) of serum vitamin B_{12} and folate were 326 (259–395) pg/ml and 7.7 (6–10) ng/ml respectively. The prevalence of vitamin B_{12} and folate deficiency amongst school age children was found as 7.4% and 1.5% respectively.
Conclusions A low prevalence of vitamin B_{12} and folate deficiencies was found amongst children aged 6–18 y living at high altitude regions in India. This is possibly due to high frequency of consumption of foods rich in vitamin B_{12} and folate.

Keywords Vitamin B_{12} deficiency · Cobalamin deficiency · Folic acid deficiency · Non vegetarian diet · Vegetarian diet · Dietary pattern

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Introduction

Low vitamin B_{12} and folate status is associated with impairment in memory and learning, lower academic scores and cognitive performance at school amongst school children [1–3]. Vitamin B_{12} and folate deficiency are also important etiological factors of megaloblastic anemia amongst children in the age group of 6–18 y. Deficiencies of vitamin B_{12} and folate occur primarily as a result of insufficient dietary intake [4]. Animal-source foods are the only natural source of vitamin B_{12}. Whereas, folate is present in high concentrations in vegetarian sources such as legumes and green leafy vegetables (GLV's).

High prevalence of vitamin B_{12} and folate deficiency have been earlier reported amongst school age children in India. Majority of these studies have been conducted in plain regions [5–10]. We do not have adequate data on the prevalence of vitamin B_{12} and folate deficiencies among school age children in the age group of 6–18 y residing at high altitude regions of Himachal Pradesh. Hence, this study was conducted to provide scientific evidence and fill the gap in the existing knowledge in this area.

Material and Methods

A community based cross-sectional study was conducted during July through December 2015 in three districts of Himachal Pradesh namely Kangra, Kullu and Shimla. Ten clusters were identified in each district using population proportionate to size sampling method. A minimum of 7 children were selected from each cluster (school) with the help of random number table.

A total of 215 children in the age group of 6–18 y studying in Government schools were included. Since the school enrollment was more than 90%, the children studying in the

school were considered as a proxy for children residing in the area.

The sample size was calculated with the presumption of prevalence of vitamin B_{12} and folate deficiency amongst school age children to be 40% each. With precision of absolute 10%, 95% confidence level and design effect of 2.0, a total sample size of 200 children was calculated for assessment of prevalence of vitamin B_{12} and folate deficiency in school age children. However, the authors included a total of 215 children for the study.

A pretested semi-structured questionnaire was administered to each child to obtain information on identification data and socio-demographic profile. Assessment of socioeconomic status (SES) was done using Kuppuswamy's SES scale for children in the age group of 12–18 y [11]. The consumption pattern of foods with high in dietary vitamin B_{12} and folate was recorded for children in the age group of 12–18 y using food frequency questionnaire [12].

Blood samples were collected by venipuncture from median cubital veins of all subjects. After collection, blood samples were centrifuged within 2 h, and serum samples were stored at −20 °C until transported to the central laboratory of All India Institute of Medical Sciences, New Delhi. The blood serum was separated and stored at −70° in screw capped serum storage vials until analysis. Serum vitamin B_{12} and folate was assessed using standard laboratory procedures (autoanalyzers). The biochemical estimation of serum vitamin B_{12} and folate was done using chemiluminescence immunoassay method. The levels of serum vitamin B_{12} and folate less than 203 pg/ml and 4 ng/ml respectively were considered as indicative of deficiency [3].

Results

A total of 215 children (107 males; 108 females) were included. Out of 215 children, 71 children were in the age group of 6–11 y and 144 children were in the age group of 12–18 y. The mean age of the male and female subjects was 12.7 ± 3.5 y and 13.4 ± 3.3 y, respectively. It was found that children belonging to the age group of 6–11 y had higher serum vitamin B_{12} ($p < 0.01$) and serum folate levels ($p = 0.099$) than those in the age group of 12–18 y (Table 1).

According to the SES, 67.4% ($n = 97$) and 32.6% ($n = 47$) subjects belonged to middle and lower SES, respectively. Children belonging to middle income group (MIG) (336.1 ± 125.0) had higher serum vitamin B_{12} levels than subjects belonging to lower income group (LIG) (310.7 ± 101.8) (Table 1).

According to the dietary pattern, 72% ($n = 104$) of the subjects were non-vegetarians and 27% ($n = 40$) were vegetarians. Non vegetarians (331.3 ± 129.9) had higher mean serum vitamin B_{12} levels than vegetarians (318.8 ± 80.5). Whereas, vegetarians (9.0 ± 4.5) had higher mean serum folate levels as compared to non vegetarians (7.6 ± 2.8) ($p < 0.05$) (Table 1).

Animal products such as red meat, poultry, fish and eggs were consumed atleast once a week by 62% ($n = 60$) of the non vegetarian subjects. Milk was consumed daily by 50% ($n = 72$) subjects. It was found that subjects who consumed fish, poultry and meat products atleast once a week had higher vitamin B_{12} levels those who consumed them irregularly. Higher serum vitamin B_{12} levels were observed amongst subjects who consumed eggs and milk daily compared to subjects who consumed them less frequently (Table 1).

Pulses were consumed by 40% ($n = 57$) subjects daily. Fifty one percent ($n = 73$) subjects consumed GLV's atleast once a week. Subjects who consumed pulses and GLV's with higher frequency had higher serum folate levels (Table 1).

Estimation of vitamin B_{12} and folate could be undertaken in the serum samples of 215 and 200 children, respectively. The median levels (interquartile range) of serum vitamin B_{12} and folate were found to be 326 (259–395) pg/ml and 7.7 (6–10) ng/ml, respectively. It was found that 7.4% ($n = 16$) (95% CI: 4.7–12.4%) subjects had vitamin B_{12} deficiency (serum vitamin $B_{12} < 203$ pg/ml or <150 pmol/L). Folate deficiency was prevalent in 1.5% ($n = 3$) (95% CI: 0.31–4.3%) subjects (serum folate levels <4 ng/ml or <10 nmol/L).

Statistical Package for Social Sciences (SPSS) version 20.0 was used for statistical analysis of the data. Quantitative data (serum vitamin B_{12} and folate) values were expressed in mean ± SD; quantitative data was expressed in frequency. T test and ANOVA were used to establish the association between different parameters with serum vitamin B_{12} and folate levels. The $P < 0.05$ was considered as statistically significant.

Discussion

High prevalence of vitamin B_{12} deficiency in the range of 12–51% has been reported amongst school age children residing in plain regions of India [5–9]. The present study showed low prevalence of vitamin B_{12} deficiency (7.4%) amongst school age children living at high altitude region. An earlier study conducted amongst 499 children in the age group of 6–10 y living in hilly regions of Uttrakhand also reported low prevalence of 1.2% deficiency (serum vitamin $B_{12} < 150$ pmol/L) due to high consumption of animal products [13]. Lower prevalence of vitamin B_{12} deficiency observed amongst school age children may possibly be due to two reasons: i) Majority (72.2%) of the subjects were non vegetarians and ii) WHO 2008 cutoffs (serum vitamin $B_{12} < 150$ pmol/L) utilized for classification of children with vitamin B_{12} deficiency which are lower than the earlier cut offs of <300 pmol/L [4].

Previous studies have suggested that vegetarians have a higher risk of developing vitamin B_{12} deficiency than those consuming non vegetarian diets due to reduced bioavailability

Table 1 Relationship between serum vitamin B_{12} and folate levels with different parameters amongst children aged 6–18 y

S.No.	Parameters	Serum vitamin B_{12}		*p* value	Serum folate		*p* value
		n	Mean ± SD		n	Mean ± SD	
1.	Age group						
	6–11 y	71	383.5 ± 158.2	<0.01	65	8.8 ± 3.0	0.0995
	12–18 y	144	327.8 ± 118.1		135	8.0 ± 3.4	
2.	Gender						
	Male	107	335.1 ± 127.7	0.2293	97	8.5 ± 3.7	0.3065
	Female	108	357.2 ± 141.5		103	8.0 ± 2.9	
3.	Socioeconomic status*						
	Upper	0	NIL	0.2277	0	NIL	0.3021
	Middle	97	336.1 ± 125.0		91	7.8 ± 3.1	
	Lower	47	310.7 ± 101.8		44	8.4 ± 3.9	
4.	Dietary pattern*						
	Vegetarian	40	318.8 ± 80.5	0.5710	38	9.0 ± 4.5	<0.05
	Non vegetarian	104	331.3 ± 129.9		97	7.6 ± 2.8	
5.	Consumption of fish, poultry and meat*						
	Daily	0	NIL	0.4955	0	NIL	0.2567
	1–6 d a wk	20	344.6 ± 149.1		17	8.9 ± 4.2	
	Irregular	124	325.1 ± 112.9		118	7.9 ± 3.3	
6.	Consumption of eggs*						
	Daily	5	343.4 ± 163.7	0.9170	5	7.9 ± 3.1	0.9977
	1–6 d a wk	49	331.2 ± 126.3		44	8.0 ± 3.3	
	Irregular	90	325.1 ± 112.2		86	8.0 ± 3.5	
7.	Consumption of milk*						
	Daily	72	333.2 ± 126.7	0.8526	68	8.0 ± 3.6	0.4339
	1–6 d a wk	27	319.6 ± 77.3		24	8.7 ± 4.1	
	Irregular	45	324.1 ± 125.9		43	7.5 ± 2.7	
8.	Consumption of milk products*						
	Daily	45	317.9 ± 111.1	0.6216	41	9.1 ± 4.5	0.2383
	1–6 d a wk	77	327.8 ± 105.9		72	7.6 ± 2.9	
	Irregular	22	348.0 ± 167.3		22	7.3 ± 2.3	
9.	Consumption of pulses*						
	Daily	57	320.0 ± 130.2	0.5254	54	8.4 ± 3.9	0.3998
	1–6 d a wk	87	332.9 ± 110.0		81	7.8 ± 3.1	
	Irregular	0	NIL		0	NIL	
10.	Consumption of green leafy vegetables*						
	Daily	0	NIL	0.3354	0	NIL	0.1594
	1–6 d a wk	73	318.4 ± 118.0		67	8.4 ± 3.9	
	Irregular	71	337.5 ± 118.4		68	7.6 ± 2.8	

*Serum vitamin B_{12}: Information was provided by 144 subjects in the age group of 12–18 y

Serum folate: Information was provided by 135 subjects in the age group of 12–18 y

of vitamin B_{12} from plant sources [14–23]. The present findings are consistent with the previous evidence showing that non vegetarians have higher serum vitamin B_{12} levels than vegetarians. In addition, serum vitamin B_{12} levels were higher in subjects who consumed animal products such as fish, meat, poultry, eggs and milk more frequently. Association between dietary intake of vitamin B_{12} from animal products and serum vitamin B_{12} levels have been documented by similar studies [12–20].

A German study showed that 40% of subjects who consumed a vegan diet for more than 5 y had vitamin B_{12} deficiency and had 1.8 times higher risk of developing deficiency than non vegans [24]. A similar study conducted in India documented that vegetarians had 4.4 times higher risk of

vitamin B_{12} deficiency than non vegetarians [25]. Lower serum levels of vitamin B_{12} associated with vegetarian diets have also shown to result in hyperhomocysteinemia amongst school age children [26, 27].

It was observed that economic status may also be an important determinant of vitamin B_{12} status as the subjects belonging to LIG had lower serum B_{12} levels than the subjects in MIG. This may be due to higher cost of animal products which are not affordable by LIG.

The prevalence of folate deficiency amongst school age children was found to be 1.5%. Previous studies conducted in India have shown higher prevalence of folate deficiency of more than 40% amongst school age children [5–10, 13]. In the index study the low prevalence of folate deficiency is possibly due to regular consumption of pulses and GLV's by the subjects. It was observed that subjects who had higher frequency of consumption of pulses and GLV's had higher serum folate levels. In accordance to the present study, earlier studies have also reported relationship between dietary intake of folate and serum folate levels [14, 19, 20].

A recent study conducted amongst Indian subjects showed that vegetarian diet has significantly greater amounts of legumes, vegetables and higher folate content than the non vegetarian diets [14]. The index study showed similar results as vegetarians ($p < 0.05$) had significantly higher serum folate levels than non vegetarians due to higher frequency of consumption of pulses and GLV's by them.

The findings of the present study revealed low prevalence of vitamin B_{12} and folate deficiencies amongst children aged 6–18 y living at high altitude regions in India. This was possibly due to high frequency of consumption of foods rich in vitamin B_{12} and folate by the subjects. Therefore, dietary interventions including promotion of regular consumption of foods with high vitamin B_{12} and folate in the daily dietaries may be seen as a potential strategy for improving vitamin status of the population. More studies need to undertaken for assessing vitamin B_{12} and folate deficiency amongst children in other regions of the country.

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Contributions AG: Literature review, content developing, manuscript development; UK: Concept, literature review, content developing, manuscript development and will act as guarantor for the paper; LR: Biochemical estimations; RMP: Data analysis and interpretation; CPY: Data analysis and interpretation.

Compliance with Ethical Standards

Conflict of Interest None.

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