Cobalamin and Folate Deficiencies among Children in the Age Group of 12-59 Months in India

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Background: Anemia is a major public health problem among children under 5 years of age in India. Cobalamin and folate deficiencies play an important role in the etiology of anemia. This study was done to assess the prevalence of cobalamin and folate deficiencies among children in the age group of 12-59 months.

Methods: A community-based cross-sectional study was conducted. A total of 470 children were included. Non-fasting venous blood samples were collected from each child for the estimation of serum cobalamin and folate levels. Pattern of dietary consumption of the each child was assessed with the help of the food frequency questionnaire (FFQ) method.

Results: The median levels (interquartile range) of serum cobalamin (n = 469) and folate (n = 416) were found to be 275 (202-427) pg/ml and 3.02 (2.02-4.94) ng/ml, respectively. The overall prevalence of cobalamin and folate deficiencies was found to be 180/469 [38.4%; 95% Confidence Interval (CI): 34.1-42.8%] and 263/416 (63.2%; 95% CI: 58.5-67.7%), respectively.

Conclusions: A high prevalence of cobalamin and folate deficiencies was found in children under 5 years of age.

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Key words: anemia, folic acid deficiency, vitamin B_{12} deficiency

At a Glance Commentary

Scientific background of the subject

Anemia and micronutrient deficiencies among children continue to be major public health challenges in most developing countries. There is lack of data on the status of cobalamin and folate deficiencies among children in the age group of 12-59 months in India. There are also only a few studies available in the international scenario.

What this study adds to the field

This study adds to the existing knowledge. It provides lead to the scientists working in the field of etiology of anemia that cobalamin and folate deficiencies may be linked to anemia. This study contributes to our understanding that cobalamin and folate deficiencies may also be responsible in the causation of anemia. It suggests policy makers and stakeholders to consider the addition of cobalamin along with folic acid to the iron tablets which are being distributed for combating anemia in the communities.

Prevalence of anemia among young children continues to remain over 70% in India. This makes it a major public health problem in the country. The National Family Health Survey (NFHS-3) conducted in the year 2005-2006 provided scientific evidence for high prevalence of anemia in all states of the country.[1] Apart from the deficiency of iron, cobalamin and folate deficiencies play an important role in the etiology of anemia among young children. There is limited scientific data available in India on the prevalence of cobalamin and folate deficiencies among children under 5 years of age. Hence, this study was conducted to provide scientific evidence in this area.

METHODS

A community-based cross-sectional study was conducted. Four hundred seventy children residing in a population catered by Integrated Child Development Scheme (ICDS) in the National Capital Territory (NCT) of Delhi were enrolled by purposive sampling. Data on socio-demographic profile, educational status of mothers (of children), family type, standard of living (SLI), and religion and caste of the children were collected through a pretested structured questionnaire. The criterion for classifying the families into different socio-economic groups was based on the physical assets available in

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the household. This had been earlier utilized by the NFHSs in India.[1] Current morbidity status of each child was assessed by diagnosing the morbid conditions such as diarrhea, fever, respiratory infection, ring worm, measles, mouth ulcer, dysentery, upper respiratory tract infection (URTI), etc. during the last 24 h at the time of the interview. The children suffering from any morbidity at the time of survey were excluded.

Pattern of dietary consumption of the each child was assessed with the help of the food frequency questionnaire (FFQ) method. This method obtains retrospective information on the pattern of consumption of the food items categorized into major food groups like cereals/grains (wheat and rice), pulses and legumes, green leafy vegetables, roots and tubers, other vegetables, fruits, milk, milk products, eggs, flesh foods, nuts and oil seeds, fats and oils, and sugar/jaggery at a defined period in the past. The common food items consumed by the children were listed so that the mothers could be facilitated to comprehend and recall the food items consumed by their children. The mother of the child was enquired about the frequency of intake of the food items of specific food groups during the last 6 months of the survey. The frequency of consumption was assessed under four categories: (1) Number of days per week (1-7 days), (2) once per 15 days, (3) once per month, and (4) never.

Collection of blood sample

Non-fasting venous blood samples were collected from each child for the estimation of serum cobalamin and folate levels. The blood samples were collected from 8 a.m. to 12 noon and transported to the field laboratory. Samples were centrifuged at 2500-3000 rpm for 10-15 min to separate serum. The serum samples were then transported to the Central Laboratory and stored at −70°C.

The serum C-reactive protein (CRP) level was estimated in each sample using high-sensitivity CRP enzyme-linked immunosorbent assay (ELISA). The blood samples with CR*P* values 8.2 mg/l and above were excluded for the estimation of serum cobalamin and folate.

Criteria for cobalamin and folate deficiencies

According the World Health Organization (WHO), serum cobalamin level of less than 203 pg/ml and serum folate level of less than 4 ng/ml are the cut-offs in children that show deficiency.[2]

The data were statistically analyzed using SPSS-22.0 (International Business Machine (IBM) Statistical Package for Social Sciences Statistics Version 22.0, Institute of Liver and Biliary Sciences, New Delhi). The variables were categorized into two to four groups depending on the variable and its response. After categorization of data, the descriptive statistics (frequency distribution and percentages) were calculated. The estimation of cobalamin and folate levels in serum was carried out in the National Accreditation Board for Testing and Calibration Laboratories (NABL) accredited laboratory, "Centre for Promotion of Nutrition Research and Training with special reference to North-East, Tribal and Inaccessible Population" of Indian Council of Medical Research (ICMR), New Delhi. Standard laboratory methods were used for the estimation of serum cobalamin and folate levels. Serum folate was determined by the radioimmunoassay (RIA) method and serum cobalamin by the radioisotopic method.[3-5] The study was ethically approved by the ethics committee of the All India Institute of Medical Sciences, New Delhi. Written informed consent was obtained from either of the parents of each child. Details of the study were given in writing and also read out aloud in front of parents (in case of illiterates) in the presence of a witness. The children of those parents who gave consent for their participation were enrolled in the study.

RESULTS

A total of 470 children were included in the study. The proportion of male and female children was almost equal. Estimation of cobalamin and folate was done in the serum samples of 469 and 416 children, respectively. The median levels (interquartile range) of serum cobalamin and folate were found to be 275 (202-427) pg/ml and 3.02 (2.02-4.94) ng/ml, respectively. The median folate levels were insignificantly low among the cobalamin-deficient children. However, the median cobalamin levels were found to be insignificantly high among the folate-deficient children ($p > 0.05$) [Table 1].

In the present study, cobalamin deficiency was found in 180/416 (38.38%) children (36.8% among boys and 40% among girls) [Table 2]. The subjects belonging to low

Table 1: Serum cobalamin (*n*=469) and folate (*n*=416) levels (median with interquartile range) among children aged 12-59 months

Serum levels	Deficient in cobalamin	Not deficient in cobalamin	*p* value*	Deficient in folate	Not deficient in folate	*p* value*
Cobalamin levels (pg/ml)						
n				263	153	
Median (interquartile range)				247 (176-386)	242 (164-397)	0.58
Folate levels (ng/ml)						
n	180	289				
Median (interquartile range)	2.99 (1.92-4.69)	3.02 (2.04-5.14)	0.47			

*Mann-Whitney U test

SLI (43.7%) were more deficient when compared to those belonging to medium SLI (37.6%) and high SLI (18.6%) ($p < 0.001$). A high deficiency of cobalamin was found among children belonging to Schedule Caste (SC) (50%) and Other Backward Caste (OBC) (48.6%) as compared to those of Schedule Tribe (ST) (39.7%) and other castes (15.8%) ($p < 0.001$). Cobalamin deficiency was significantly lower in children who consumed flesh foods and eggs more frequently ($p < 0.05$). Cobalamin deficiency was more prevalent in vegetarian subjects (50.4%) in comparison to non-vegetarian subjects (33.8%) ($p = 0.001$). On multiple logistic regression analysis, it was found that children belonging to low SLI, SC, ST, and OBC, and not consuming flesh in diet were more deficient [Table 3].

In the present study, folate deficiency was found in 263/416 (63.22%) children. It was equally prevalent among boys (65.4%) and girls (60.9%) [Table 4]. The subjects belonging to high SLI (75.4%) were more folate deficient when compared to those belonging to medium SLI (62.4%) and low SLI (60.6%) ($p = 0.11$). About 50% of SC subjects were folate deficient. Folate deficiency was less amongst children consuming foods regularly with low sugar, more eggs, less fats and oils, less milk and milk products, and fewer nuts. Folate deficiency was more prevalent in vegetarian (74.3%) subjects as compared to non-vegetarian (59.1%) subjects ($p = 0.004$). On multiple logistic regression analysis, it was found that children on vegetarian diet with high consumption of milk, fats, and oils were more deficient [Table 5].

DISCUSSION

The present study was conducted to assess the prevalence of cobalamin and folate deficiencies amongst children in the age group of 12-59 months. The overall prevalence of cobalamin deficiency was found to be 38.38% (95% CI: 34.1-42.8%). Two previous studies from India have reported lower prevalence of cobalamin deficiency (9% and 14.4%, respectively) as compared with the results of present study.[6,7] However, higher prevalence of cobalamin deficiency of 50% has been reported in another earlier study.[8]

The prevalence of folate deficiency was found to be 63.22% (95% CI: 58.5-67.7%). An earlier study conducted amongst children of similar age group documented a folate deficiency of 33%.[6] This variation could be due to the different cut-offs utilized for classification of children with folate deficiency. Earlier studies have also documented a prevalence of 62% and 65%.[7,8]

In the present study, 13.5% children had concomitant prevalence of cyanocobalamin and folate deficiencies. This finding indicates that cobalamin and folate deficiencies could be independent etiological factors in the causation of nutritional anemia amongst young children.

The present study was undertaken in children who consumed a cereal-based diet which provides about 90% of the calories. The cobalamin and folate deficiencies in

Table 2: Characteristics of cyanocobalamin deficiency amongst children aged 12-59 months (n=469)

Characteristics	Non-deficient (n=289)	Deficient (n=180)	Total (n=469)	p value*
Gender				
Male	151 (63.2)	88 (36.8)	239 (51.0)	0.507
Female	138 (60.0)	92 (40.0)	230 (49.0)	
Caste				
Schedule tribe	76 (60.3)	50 (39.7)	126 (26.9)	<0.001
Schedule caste	28 (50.0)	28 (50.0)	56 (11.9)	
Other backward caste	89 (51.4)	84 (48.6)	173 (36.9)	
Others	96 (84.2)	18 (15.8)	114 (24.3)	
Religion				
Hindu	244 (58.5)	173 (41.5)	417 (88.9)	<0.001
Muslim	40 (85.1)	7 (14.9)	47 (10.0)	
Others	5 (100.0)	0 (0.0)	5 (1.1)	
Standard of living (SLI)				
Lower	138 (56.3)	107 (43.7)	245 (52.2)	0.002
Middle	103 (62.4)	62 (37.6)	165 (35.2)	
High	48 (81.4)	11 (18.6)	59 (12.6)	
Dietary habits				
Vegetarian	64 (49.6)	65 (50.4)	129 (27.5)	0.001
Non-vegetarian	225 (66.2)	115 (33.8)	340 (72.5)	
Consumption of flesh				
7 days a week	9 (100.0)	0 (0.0)	9 (1.9)	0.001
1-6 days a week	155 (69.2)	69 (30.8)	224 (47.8)	
Never	125 (53.0)	111 (47.0)	236 (50.3)	
Consumption of milk				
7 days a week	176 (60.9)	113 (39.1)	289 (61.6)	0.899
1-6 days a week	42 (61.8)	26 (38.2)	68 (14.5)	
Never	71 (63.4)	41 (36.6)	112 (23.9)	
Consumption of milk products				
7 days a week	55 (54.5)	46 (45.5)	101 (21.5)	0.084
1-6 days a week	146 (66.7)	73 (33.3)	219 (46.7)	
Never	88 (59.1)	61 (40.9)	149 (31.8)	
Consumption of fats and oils				
7 days a week	120 (64.9)	65 (35.1)	185 (39.4)	0.124
1-6 days a week	127 (62.6)	76 (37.4)	203 (43.3)	
Never	42 (51.9)	39 (48.1)	81 (17.3)	
Consumption of nuts				
7 days a week	23 (74.2)	8 (25.8)	31 (6.6)	0.148
1-6 days a week	160 (63.2)	93 (36.8)	253 (53.9)	
Never	106 (57.3)	79 (42.7)	185 (39.4)	
Consumption of eggs				
7 days a week	20 (87.0)	3 (13.0)	23 (4.9)	0.006
1-6 days a week	143 (64.7)	78 (35.3)	221 (47.1)	
Never	126 (56.0)	99 (44.0)	225 (48.0)	
Consumption of sugar				
7 days a week	226 (62.1)	138 (37.9)	364 (77.6)	0.901
1-6 days a week	33 (61.1)	21 (38.9)	54 (11.5)	

Contd...

Table 2: Contd...

Characteristics	Non-deficient (*n*=289)	Deficient (*n*=180)	Total (*n*=469)	*p* value*
Never	30 (58.8)	21 (41.2)	51 (10.9)	
Consumption of green leafy vegetables				
7 days a week	62 (61.4)	39 (38.6)	101 (21.5)	0.684
1-6 days a week	181 (62.8)	107 (37.2)	288 (61.4)	
Never	46 (57.5)	34 (42.5)	80 (17.1)	

*Chi-square test, figures in parenthesis are percentages

Table 3: Independent risk factors for cobalamin deficiency as per forward conditional multiple logistic regression analysis

Parameters	β-coefficient	Odds ratio (95% CI)	*p* value
Consumption of flesh			
Never		Reference category	
1-6 or 7 days a week	0.493	1.637 (1.091-2.455)	0.017
Caste			
Others (general caste)		Reference category	
Schedule tribe	0.957	2.605 (1.360-4.989)	0.004
Schedule caste	1.331	3.784 (1.760-8.135)	0.001
Other backward caste	1.363	3.908 (2.126-7.184)	<0.001
Standard of living (SLI)			
High SLI		Reference category	
Middle SLI	0.736	2.087 (0.973-4.475)	0.059
Low SLI	0.942	2.564 (1.202–5.470)	0.015

Abbreviation: CI: Confidence interval

Table 4: Characteristics of folate deficiency amongst children aged 12-59 months (*n*=416)

Characteristics	Non-deficient (*n*=153)	Deficient (*n*=263)	Total (*n*=416)	*p* value*
Gender				
Male	74 (34.6)	140 (65.4)	214 (51.4)	0.338
Female	79 (39.1)	123 (60.9)	202 (48.6)	
Caste				
Schedule tribe	43 (37.7)	71 (62.3)	114 (27.4)	0.023
Schedule caste	26 (50.0)	26 (50.0)	52 (12.5)	
Other backward caste	58 (38.9)	91 (61.1)	149 (35.8)	
Others	26 (25.7)	75 (74.3)	101 (24.3)	
Religion				
Hindu	142 (38.4)	228 (61.6)	370 (88.9)	0.080
Muslim	11 (26.8)	30 (73.2)	41 (9.9)	
Others	0 (0.0)	5 (100.0)	5 (1.2)	
Standard of living (SLI)				
Lower SLI	86 (39.4)	132(60.6)	218 (52.4)	0.113
Middle SLI	53 (37.6)	88 (62.4)	141 (33.9)	
High SLI	14 (24.6)	43 (75.4)	57 (13.7)	
Dietary habits				
Vegetarian	29 (25.7)	84 (74.3)	113 (27.2)	0.004
Non-vegetarian	124 (40.9)	179 (59.1)	303 (72.8)	
Consumption of flesh				
7 days a week	1 (11.1)	8 (88.9)	9 (2.2)	0.271
1-6 days a week	74 (37.6)	123 (62.4)	197 (47.4)	
Never	78 (37.1)	132 (62.9)	210 (50.5)	

Contd...

Table 4: Contd...

Characteristics	Non-deficient (*n*=153)	Deficient (*n*=263)	Total (*n*=416)	*p* value*
Consumption of milk				
7 days a week	84 (33.9)	164 (66.1)	248 (59.6)	0.029
1-6 days a week	20 (30.8)	45 (69.2)	65 (15.6)	
Never	49 (47.6)	54 (52.4)	103 (24.8)	
Consumption of milk products				
7 days a week	26 (29.9)	61 (70.1)	87 (20.9)	0.005
1-6 days a week	65 (32.5)	135 (67.5)	200 (48.1)	
Never	62 (48.1)	67 (51.9)	129 (31.0)	
Consumption of fats and oils				
7 days a week	35 (22.0)	124 (78.0)	159 (38.2	<0.001
1-6 days a week	71 (38.2)	115 (61.8)	186 (44.7)	
Never	47 (66.2)	24 (33.8)	71 (17.1)	
Consumption of nuts				
7 days a week	11 (37.9)	18 (62.1)	29 (7.0)	0.001
1-6 days a week	66 (29.2)	160 (70.8)	226 (54.3)	
7 days a week	36 (41.4)	51 (58.6)	87 (20.9)	<0.001
1-6 days a week	77 (29.7)	182 (70.3)	259 (62.3)	
Never	40 (57.1)	30 (42.9)	70 (16.8)	

*Chi-square test, figures in parenthesis are percentages

Table 5: Independent risk factors for folate deficiency as per forward conditional multiple logistic regression analysis

Parameters	β-coefficient	Odds ratio (95% CI)	*p* value
Dietary habits			
Non-vegetarian		Reference category	
Vegetarian	0.736	2.15 (1.27-3.62)	0.004
Consumption of milk			
Never		Reference category	
1-6 days a week	0.865	2.38 (1.16-4.89)	0.019
7 days a week	0.520	1.68 (1.01-2.83)	0.049
Consumption of fats and oils			
Never		Reference category	
1-6 days a week	1.097	2.99 (1.63-5.53)	<0.001
7 days a week	2.002	7.40 (3.89-14.05)	<0.001

Abbreviation: CI: Confidence interval

children could possibly due to lack of variety in the foods consumed and an overall inadequate food intake.

Conclusion

The findings of the present study revealed the high prevalence of cobalamin and folate deficiencies amongst children under 5 years of age and belonging to low income group in India. In view of the role of cobalamin and folate deficiencies in anemia, there is a need to have more scientific evidence on the prevalence of cobalamin and folate deficiencies in different regions of India so that public health intervention can be initiated at the national level.

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