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Research Article

## Evaluation of vitamin B12 deficiency in preschool children-A pilot study in east Delhi

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### Abstract

Anemia is an important health concern worldwide, particularly in tropical and sub-tropical countries such as in India. The objective of the present study was to determine the prevalence of vitamin B12 deficiency in pre-school children of 6 to 60 months (5 years). **Methods:** Vitamin B12 assays on 528 children between 6 months to 5 years over a period of 2 years were reviewed. Individuals were considered deficient if vitamin B12 levels < 200 pg/mL and border line deficient if levels were < 350 pg/mL. **Results:** Vitamin B12 deficiency was observed in 44.1% of the paediatric population in age group of 6-60 months. 52.5% of children were found deficient when cut-off was taken < 200 pg/mL. Mean value of B12 observed in the study population was  $188.96 \pm 93.1$  pg/mL. No difference in B12 levels were observed between male and female children. **Conclusion:** Vitamin B12 deficiency is prevalent in population studied and serum Vitamin B12 status is an important predictor for plasma Hb concentration. Thus the low level of Vitamin B12 may be a cause for high anemia prevalence. So, improving the status of this micronutrient by dietary modification and supplementation may reduce the burden of childhood anemia in India.

**Keywords:** Anemia, vitamin B12 deficiency, children, India, micronutrient

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### 1. Introduction

Nutritional anaemia in children contributes to a significant public health problem worldwide. Its occurrence is more commonly observed amongst the under-nourished and malnourished societies of tropical and sub-tropical countries. According to the WHO estimates 1.62 billion people are anaemic amongst which 47.4% belong to the pre-school age group. Of the 293 million anaemic children, 89 million are said to reside in India [1]. Vitamin B12 is essential for the synthesis of S-adenosyl methionine which is involved in the metabolism of different proteins, phospholipids and neurotransmitters [2]. Its deficiency may present in multiple ways, from haematological manifestations such as megaloblastic anaemia to neurological ones such as subacute combined degeneration of the spinal cord [3, 4]. Vitamin B12 deficiency in children may present as failure to thrive, movement disorders, psychomotor developmental delay and regression [5]. Strict

vegetarian diets may be associated with vitamin B12 deficiency, both in the mother and in the newborn, because the demand for this vitamin increases during pregnancy and lactation [6]. Even though the human body can store Vitamin B12 for up to five years, its deficiency is still quite common. Apart from causing neuro-psychiatric symptoms, deficiency may also lead to hyperhomocystinemia and methylmalonic academia which may lead to severe health effects. The estimation of homocysteine and methyl malonic acid may help in the diagnosis of B12 deficiency but the use of serum Vitamin B12 level for estimation is still the most extensively applied investigation for all practical purposes [7]. There is paucity of data on the prevalence of Vitamin B12 deficiency in pre-school going children in India. The present study was therefore aimed to assess the prevalence of B12 deficiency amongst pre-school children 6 to 60 months (5 years) of low socio-economic status of an urban community belonging to East Delhi.

## 2. Materials and methods

The present retrospective study was conducted in the Department of Biochemistry at Chacha Nehru Bal Chikitsalya Hospital, New Delhi. All cases in which vitamin B12 assays had been done in our laboratory from 1<sup>st</sup> January 2012 till 31<sup>st</sup> December 2013 were retrieved. Data on Vitamin B12 level, age and sex were collected. Serum levels of Vitamin B12 (normal range 180-914 pg/mL) were estimated on venous blood sample collected under aseptic conditions using chemiluminescence method on Access-2 Beckman Coulter analyser. The anaemic patients who had, vitamin B12 level < 200pg/ml were diagnosed as vitamin B12 deficient whereas at levels < 350 pg/mL were taken as border line deficient. Records of 1196 children in the age group of 6 to 60 months (5 years) where B12 levels were assayed were screened. Out of these, 528 children had vitamin B12 level < 350 pg/mL and were included for analysis in the present study. Results of the study were expressed as percentage. One-way analysis of variance (ANOVA) was used to compare vitamin B12 levels in children of different age groups. Student's t test was used for comparison between vitamin B12 values in male and female children. Calculations were done using SPSS software, version 12.0 for Windows.

## 3. Results

Out of the 1196 children in the age group of 6-60 months (5years) screened during the 2 year period of our study, 528 children (44.1%) had vitamin B12 deficiency < 350 pg/mL. Amongst these 528 children, 66 % were male whereas 34 % children were female with a sex ratio of 1.93:1. Majority of the anaemic children belonged to the age group of 6-24 months (~34%) and only 8% were observed to be in the age group of 37-48 months as shown in Table No.1. The average age of the male children in our study was  $22.9 \pm 15.5$  months and female  $24.3 \pm 15.8$  months. Out of the total 528 subjects it was observed that 277 (52.5 %) children had vitamin B12 deficiency with cut off levels < 200 pg/mL. On further distributing the anaemic children by age, it was observed that the highest prevalence of vitamin B12 deficiency (68%) was seen in children belonging to the age group of 6-24 months, amongst these 189 (53 %) were identified as being deficient with vitamin B12 cut off level as < 200 pg/mL. In contrast a low

prevalence of vitamin B12 deficiency (17%) was observed in the 37-60 months age group with 55.6% of these children having vitamin B12 < 200pg/mL (Table No.2). Vitamin B12 levels were estimated in different age groups of the children under study. It was observed that infants had the lowest levels of vitamin B12 concentration with a mean of  $164.49 \pm 92.55$  pg/mL. A significant difference in mean B12 levels was observed in between children of different age groups ( $p < 0.001$ ) as shown in Table No.3.

Table No.1:- Age and Sex distribution of 528 children (6-60 months) studied for Vitamin B12 deficiency.

Age (months)	Girls	Boys	Total
6-12 mo	58	124	182 (34.5%)
13-24 mo	59	122	181 (34.3%)
25-36 mo	33	44	77 (14.5 %)
37-48 mo	11	31	42 (8%)
49- 60 mo	19	27	46 (8.7%)
Total	180	348	528

Table No. 2:- Age wise distribution of anaemic children according to Vitamin B12 levels.

Characteristics	< 200 pg/mL (n = 277)	< 350 pg/mL (n = 528)
AGE (months)	N (%)	N (%)
6-12	100 (56.2 %)	178 (33.7 %)
13-24	89 (50 %)	179 (33.9 %)
25-36	38 (47 %)	81 (15.3 %)
37-48	24 (58.5 %)	41 (7.8 %)
49-60	26 (9.4 %)	49 (9.3 %)

Table No. 3:- Mean Vitamin B12 levels in anaemic children of different age groups

Characteristics AGE (months)	Vitamin B12 level (Mean $\pm$ SD) pg/mL
6-12	$164.49 \pm 92.55$
13-24	$197.25 \pm 93.44$
25-36	$224.87 \pm 78.15$
37-48	$196.78 \pm 101.47$
49-60	$184.52 \pm 86.14^*$

Table No.4 depicts the sex distribution of the anaemic subjects in accordance to vitamin B12 levels. Mean  $\pm$  SD levels of vitamin B12 in 528 children studied were observed to be  $188.96 \pm 93.1$ pg/mL. Boys had a mean value of  $193.17 \pm$

Table No. 4:- Sex distribution of anemic children according to Vitamin B12 levels

Characteristics	< 200 pg/mL		200-350 pg/mL		Overall Total Levels (upto 350 pg/mL)	
	N (%)	Mean ± SD	N (%)	Mean ± SD	N (%)	Mean ± SD
Sex						
Male	176 (33.3)	119.44 ± 54.5	171 (32.4)	269.93 ± 45.6	347 (65.7)	193.17 ± 90.6
Female	101 (19.1)	106.95 ± 57.1	80 (15.2)	274.28 ± 40.9	181 (34.3)	180.91 ± 97.4
Total	277 (52.5)	114.9 ± 55.7	251 (47.5)	271.32 ± 44.1	528 (100)	188.96 ± 93.1

90.6 pg/mL whereas girls had a mean value of 180.91 ± 97.4 pg/mL. If vitamin B12 < 200 pg/mL was taken as the cut-off for deficiency state than total 277 subjects out of 528 (52.5%) turned out to have B12 deficiency. Amongst which 176 (33.3%) were boys with mean vitamin B12 level of 119.44 ± 54.5 pg/mL and 101 (19.1%) were girls with mean of 106.95 ± 57.1 pg/mL. No significant difference was observed between the two deficient groups ( $p = 0.072$ ). It was observed that 251 (47.5 %) children out of 528 had border line deficiency. Using this cut-off value 171 (32.4%) boys and 80 (15.2%) girls were included in this category. There was no significant difference in the B12 levels in the males and females ( $269.93 \pm 45.6$  v/s  $274.28 \pm 40.9$  respectively,  $p = 0.467$ ).

#### 4. Discussion

The deficiency of Vitamin B12 is not as uncommon as it was previously thought. In most western countries the disease is more commonly observed in the elder population with malabsorption being the most important cause. However in India the disease affects all age groups with inadequate diet being the foremost cause [8]. The disease state has wide presentation and can range from subtle and slight cognitive impairment to severe neuro-psychiatric derangement. Hence early detection is important for preventing irreversible damage especially in children. Dietary deficiency of Vitamin B12 is an important public health problem of our country [9] as observed in our study. The mean Vitamin B12 in our subject population was 188.96 ± 93.1 pg/mL, which was on the lower side of the normal reference range. In the present study the prevalence of vitamin B12 deficiency was 44.1%. Out of the 528 children from 6 months to 5 years it was observed that 52.5% (277 out of 528) had vitamin B12 levels < 200pg/mL whereas borderline deficient cases (< 350 pg/mL) made

up 47.5% of the total cases. Our findings are consistent with various other studies. In a study by Sarode et al [10] from Chandigarh, B12 deficiency was reported in nearly 85% cases with megaloblastic anemia (adults included). In a study by Gomber and his co-workers a prevalence of 36.6% was reported in pre-school children [11]. Similar reports have been observed by Saraya et al [12], Singla and his co-workers [13], Chandra et al [14] and Sarode et al [15]. Our study showed B12 deficiency to be more predominant in children from 6 -12 months with a prevalence of 56.2% in comparison to other age groups. The mean B12 level was also found to be lowest ( $164.49 \pm 92.55$  pg/mL) in this age group.

The main cause of B12 deficiency in pediatric population is maternal deficiency leading to decrease in stores at birth and its consequences. In a review by Stabler and Allen, B12 deficiency was highlighted as a worldwide problem with breast-fed infants of B12 deficient mothers being at the highest risk [16]. Breast feeding for a prolonged period (sometimes 3-5 years) leads to vitamin B12 deficiency as the breast milk content for B12 in these mothers is far below normal. Cobalamin content of breast milk is also found to be lower in vegetarian mothers and correlates positively with their serum levels [17, 18]. Dietary habits were not documented in the present study, however in previous studies conducted in this population most families were vegetarian [19]. Taneja and his co-workers in their North Indian population based study observed a decrease in the median cobalamin concentration in children breast-fed in the 6-11mth age group in comparison to non-breast fed children of same age group [20]. For smaller children dietary intake is limited during the weaning period due to limited variety of micronutrients which eventually reduces their body stores and serum levels [21]. In the present study Vitamin B12 estimation was the first line test. Estimation of metabolites such as methylmalonic acid and

homocysteine can be more sensitive for the diagnosis of vitamin B12 deficiency [20, 22, 23]. There are few limitations in our study firstly since we have only analysed vitamin B12 levels of our patients, those with subclinical deficiency (elevated homocysteine and methylmalonic acid with normal B12 levels) might be missed out. Secondly since it is a hospital based study the population cannot be representative of all north Indian children. Lastly dietary information of the children and parents could not be retrieved. Despite these limitations the prevalence observed in the present study is highly significant and consistent with other studies which address the problem of vitamin B12 deficiency. Further population based studies are required to validate our findings.

## Conclusion

Anemia is prevalent in population studied. Vitamin B12 status is an important predictor for plasma Hb concentration. Improving the status of this micronutrient may reduce the burden of childhood anemia in India. Remedial measures such as dietary modification, supplementation and fortification of specific foods should be enforced.

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