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# Status of Vitamin B12, Zinc, Copper, Selenium, Manganese, Molybdenum and Cobalt in Severe Acute Malnutrition

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## Abstract

**Objectives** To define the vitamin B12 levels and other micronutrients status in severe acute malnutrition (SAM) children.

**Methods** This was a prospective hospital based cross-sectional study. Inclusion criteria: Children with severe acute malnutrition as per WHO criteria. Exclusion criteria: (i) Pernicious anemia (ii) Autoimmune gastritis (iii) SAM children on exclusive vitamin B12 supplementation. All enrolled children underwent a detailed clinical history, general physical examination with more emphasis on clinical features of vitamin B12 and other micronutrients deficiencies. Three ml of venous blood was collected to estimate vitamin B12 and other micronutrients. Primary outcome was percentage of deficiency of serum vitamin B12, zinc, copper, selenium, manganese, molybdenum and cobalt in SAM children.

**Results** Fifty children were included in the study. The mean age of children was  $15.60 \pm 12.90$  mo with male to female ratio 0.85:1. The common clinical presentation in order of frequency were upper respiratory infection (URI) symptoms 35 (70%), hepatomegaly 24 (48%), Hyperpigmentation 17 (34%), angular cheilitis 14 (28%), tremors 11 (22%), edema 07 (14%), and hypotonia 05 (10%). Anemia was found in 44 (88%) children. Prevalence of vitamin B12 deficiency was 34%. Other micronutrient deficiencies observed were cobalt 24 (100%), copper 05 (12%), zinc 04 (9.5%), and molybdenum 03 (12.5%). No statistical significance was found between clinical symptoms and levels of vitamin B12 with different age and sex.

**Conclusions** Prevalence of low vitamin B12 and cobalt were more common than other micronutrients.

**Keywords** SAM · Tremors · Micronutrients · Vitamin B12 · Zinc · Cobalt · Copper

## Introduction

Malnutrition in children is common in the developing countries and management continues to be a challenging issue. Prevalence of severe acute malnutrition (SAM) in many states of India is still high [1]. Anemia and malnutrition affects especially young children across all parts of the world. Globally, 40% of children less than five years of age are found to have anemia. Micronutrient deficiencies particularly iron, folate and vitamin B12 deficiency constitute, the most common causes of anemia [2]. In SAM, prevalence of anemia is high and the common cause found is iron deficiency [3, 4].

In the growth and development of children, micronutrients play a pivotal role being the components of numerous enzymes and protein synthesis [5, 6]. Micronutrients

are substances, needed in small amounts for normal functions of the human body [7]. Iodine, copper, iron manganese, zinc, selenium, cobalt and molybdenum are common micronutrients. Micronutrient deficiencies impair cognition and psychomotor aspects of child development [8]. As per Golden theory of Kwashiorkor, free radical injury plays a role in pathogenesis of the disease. Micronutrients are components of antioxidants, and this necessitates measurement of micronutrients in malnourished children to improve our evidence based management [9]. Though there is not much Indian data on status of micronutrients, however recent studies observed that deficiency does exist, especially of vitamin B12 [10]. Data on micronutrient /or trace elements status in India in SAM children are limited [11]. Hence it is important to know their serum levels to improve the management in these children.

Vitamin B12 deficiency is a well known entity in exclusive breastfeed babies born to B12 deficient mothers. A study done in rural India showed that in up to one-third of women, vitamin B12 stores are depleted [12]. A study

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by Yaikhomba et al. in SAM children, found 34% children had low levels of vitamin B12 and low levels of ferritin and folate (6% each) [13]. Few authors have speculated that there may be high serum vitamin B12 levels due to inflammation in SAM. Thus the clear concept of serum levels of vitamin B12 in SAM children is not yet established.

Hence, in order to find the vitamin B12 levels and other micronutrients status in children with SAM, their plasma (zinc, copper, selenium, manganese, molybdenum and cobalt) concentration was measured on presentation to authors' hospital.

## Material and Methods

A prospective hospital based cross-sectional study done at Nutrition Rehabilitation Centre (NRC), KIMS, Hubli from May 2019 through Jan 2020. Inclusion criteria: Children with severe acute malnutrition as per WHO criteria [14]. Exclusion criteria: (i) Pernicious anemia, (ii) Auto-immune gastritis, (iii) SAM children on exclusive vitamin B12 supplementation. Pernicious and autonomic gastritis were excluded based on clinical and available previous documents. An informed written consent was taken from the parent/guardian. Ethical clearance was obtained from Institutional review board.

The relevant information of study children was obtained by using a pre-designed proforma. A detailed socio-demographic profile including parents' education, occupation, income, dietary history with special emphasis on vegetarian or non-vegetarian (child and family) food, exclusive breastfeeding, and complimentary feeding was obtained. A detailed review of past medical records, clinical history, general physical examination with more emphasis on clinical features of vitamin B12 and other micronutrient deficiencies was done. All recruited children underwent a detailed central nervous system (CNS) examination to look for neurological manifestations of B12 deficiency. Weight was measured on a digital weighing scale and recorded to the nearest 100 g, height (>2 y) was measured by stadiometer and by infantometer was used for length of the infant. Mid arm circumference (MAC) was measured using a non-stretchable tape at midpoint of left arm, midway between acromion process and olecranon process. Weight for height/length was calculated using WHO tables [14]. Measured length/height was recorded to nearest cm, mid arm circumference (MAC) to nearest mm, head circumference (HC) and chest circumference to the nearest 0.5 cm.

With due aseptic precaution, 3 ml of venous blood was collected in a test tube. The serum obtained in a plain test tube after centrifuge was used to estimate vitamin B12 and other micronutrients. The blood with anticoagulant was used for hemogram. Serum samples were stored in deep freezer at

-80°C before transported in a cold chain to the testing centre (Arogyam centre) at Navi Mumbai.

Vitamin B12 was measured using fully automated bidirectionally interfaced chemi-luminescent immuno assay by Advia Centaur XP system (Siemens, Berkely, CA, USA), serum copper levels was measured by 3,5-DIBR-PAESA colorimetric method (Elico, Hyderabad, India), serum zinc was measured by NITRO-PAPS colorimetric assay (Elico, Hyderabad, India) and other micronutrients was measured using ICP Mass Spectrometry (ThermoFisher, Waltham, MA, USA).

Anemia was defined and classified by using WHO criteria [15]. A vitamin B12 level below 203 pg/ml was taken as deficient and that above 911 pg/ml were taken as high range [16]. Zinc and copper were considered deficient, if levels were <65 µg/dl and <63.5 µg/dl, respectively [17, 18]. Other micronutrients were considered deficient if, selenium (<60 µg/L), manganese (<7.10 µg/L), molybdenum (<0.70 µg/L) and cobalt (<1.5 µg/L) [18].

Primary outcome was percentage of deficiency of serum vitamin B12, zinc, copper, selenium, manganese, molybdenum and cobalt in SAM children.

The sample size was calculated based on previous literature [19] with prevalence of 14.4% vitamin B12 deficiency in SAM children, assuming CI 95%, error rate 0.06, sample size was 140. However, sample size was restricted to 50 to meet the available budget.

MS Excel spread sheet was used for data entry. All the statistical methods were performed using SPSS version 16. Continuous variables were expressed as mean  $\pm$  standard deviation (SD) and for categorical data as percent (%). Chi-square test was used for data analysis. Results for vitamin B12, zinc, copper, selenium, manganese, molybdenum and cobalt were given as median with interquartile range (IQR). Fisher exact test was used for calculating proportion of SAM children deficient in various micronutrients at different age groups. *p* value <0.05 was considered statistically significant.

## Results

Total number of SAM children admitted during the study period were 78. Fifty children satisfied inclusion/exclusion criteria. Serum vitamin B12 levels were done for all 50 children. Serum levels of zinc and copper were done in 42 children. And serum manganese, selenium, cobalt and molybdenum were done in 24 children. Vitamin B12 was low in 17 (34%) children, cobalt in 24 (100%), copper in 05 (11.9%), zinc in 04 (9.5%), and molybdenum in 03 (12.5%) children.

**Table 1** Socio-Demographic profile of SAM children (N=50)

	Father n (%)	Mother n (%)
1. Education of parents		
Illiteracy	24 (48)	26 (52)
Class X and below	22 (44)	23 (46)
Class XI and above	04 (08)	01 (02)
2. Occupation of parents		
Agriculture laborers	28 (56)	15 (30)
Housewife	----	30 (60)
Cooli's	13 (26)	05 (10)
Others	09 (18)	-----
3. Socio-economic status*		
Class V	41 (82)	
Class IV	09 (18)	
4. Breastfeeding		
Exclusive	22 (44)	
Non-exclusive	28 (56)	
5. Complimentary foods		
Appropriate	08 (16)	
In appropriate	42 (84)	
6. Diet of parents		
Vegetarians	23 (46)	
Non-vegetarians	27 (54)	

\*B G Prasad classification

The mean age of children was  $15.60 \pm 12.90$  mo. The maximum number of children [50% (25)] were in the age group of 7–12 mo. Children aged between 1–6 mo and between 13–24 mo were 18% (9) each and those aged >24 mo were 14% (7). Male to female ratio was 0.85:1. Table 1 shows, sociodemographic profile of the SAM children. Poor education, low income of parents, low rate of exclusive breastfeeding and low appropriate complimentary feeding were observed, There was slight predominance of non vegetarian families (54% vs. 46%) noted in the study .

**Table 2** Association between clinical features and levels of vitamin B12

		Vit. B12 levels			Total	Fischer exact test
		Deficiency (<203)	Normal (203–911)	High (>911)		
Tremors	Present	5 (45.5)	5 (45.5)	1 (9.1)	11 (100)	<i>p</i> 0.709
	Absent	12 (30.8)	22 (56.4)	5 (12.8)	39 (100)	
	Total	17 (34)	27 (54)	6 (12)	50 (100)	
Hyperpigmentation	Present	7 (41.2)	7 (41.2)	3 (17.6)	17 (100)	<i>p</i> 0.391
	Absent	10 (30.3)	20 (60.6)	3 (9.1)	33 (100)	
	Total	17 (34)	27 (54)	6 (12)	50 (100)	
Angular cheilitis	Present	6 (42.9)	7 (50)	1 (7.1)	14 (100)	<i>p</i> 0.664
	Absent	11 (30.6)	20 (55.6)	5 (13.9)	36 (100)	
	Total	17 (34)	27 (54)	6 (12)	50 (100)	
Hypotonia	Present	4 (80)	1 (20)	0 (0)	5 (100)	<i>p</i> 0.101
	Absent	13 (28.9)	26 (57.8)	6 (13.3)	45 (100)	
	Total	17 (34)	27 (54)	6 (12)	50 (100)	

The common clinical presentations in order of frequency were upper respiratory infection symptoms 35 (70%), hepatomegaly 24 (48%), hyperpigmentation 17 (34%), angular cheilitis 14 (28%), tremors 11 (22%), edema 07 (14%), and hypotonia 05 (10%). No underlying congenital anomalies /or other systemic illnesses were found.

As per WHO definition of anemia, 44 (88%) of present SAM children were anemic. Among them, 11 (22%), 15 (30%), 18 (36%) had mild, moderate and severe anemia respectively. Vitamin B12 was low in 17 (34%) children and high levels of B12 were found in 06 (12%) children. Most common age group affected was 7–12 mo. The authors tried to correlate levels of vitamin B12 with age, sex [*M*=11 (47.8%), *F*=6 (22.2%)] and with various clinical features (Tables 2 and 3) but could not find any significant association.

Table 4 shows the median values with IQR of vitamin B12 and other micronutrients. The lowest serum levels of vitamin B12, zinc, copper, cobalt and molybdenum were 49 pg/ml, 43.96 µg/dl, 32.2 µg/dl, 0.29 µg/L and 0.46 µg/L respectively. The authors have not graded the deficiency severity as there were no standard definitions for grading the severity of these micro/macronutrients. Table 3 shows proportion of SAM children deficient in various micronutrients at different age groups. Among the micronutrients, cobalt was deficient in all children followed by copper 05 (12%), zinc 04 (9.5%) and molybdenum 03 (12.5%). No deficiency was found for selenium and manganese. No statistical significance was found between clinical symptoms and serum levels of all the micronutrients in different age group.

## Discussion

In recent years, severe acute malnutrition has increased from 6.4% to 7.5% in children below 60 mo of age [11]. In SAM children, occurrence of anemia is high. Anemia

**Table 3** Proportion of SAM children deficient in various micronutrients

		0–6 mo	7–12 mo	13–59 mo	Total	Fischer exact test
Vitamin B12 (pg/ml)	Deficiency (<203)	3 (33.3)	8 (32)	6 (37.5)	17 (34)	<i>p</i> 0.924
	No deficiency (≥203)	6 (66.7)	17 (68)	10 (62.5)	33 (66)	
	Total	9 (100)	25 (100)	16 (100)	50 (100)	
Zinc (µg/dl)	Deficiency (<65)	0 (0)	2 (10.5)	2 (13.3)	4 (9.5)	<i>p</i> 0.826
	No deficiency (≥65)	8 (100)	17 (89.5)	13 (86.7)	38 (90.5)	
	Total	8 (100)	19 (100)	15 (100)	42 (100)	
S. Copper (µg/dl)	Deficiency (<63.5)	0 (0)	3 (15)	2 (14.3)	5 (11.9)	<i>p</i> 0.686
	No deficiency (≥63.5)	8 (100)	17 (85)	12 (85.7)	37 (88.1)	
	Total	8 (100)	20 (100)	14 (100)	42 (100)	
Cobalt (µg/L)	Deficiency (<1.5)	3 (100)	13 (100)	8 (100)	24 (100)	NA
	No deficiency (≥1.5)	0 (0)	0 (0)	0 (0)	0 (0)	
	Total	3 (100)	13 (100)	8 (100)	24 (100)	
Molybdenum (µg/L)	Deficiency (<0.7)	0 (0)	2 (15.4)	1 (12.5)	3 (12.5)	<i>p</i> 0.99
	No deficiency (≥0.7)	3 (100)	11 (84.6)	7 (87.5)	21 (87.5)	
	Total	3 (100)	13 (100)	8 (100)	24 (100)	
Selenium (µg/L)	Deficiency (<60)	0 (0)	0 (0)	0 (0)	0 (0)	NA
	No deficiency (≥60)	3 (100)	13 (100)	8 (100)	24 (100)	
	Total	3 (100)	13 (100)	8 (100)	24 (100)	
Manganese (µg/L)	Deficiency (<7.1)	0 (0)	0 (0)	0 (0)	0 (0)	NA
	No deficiency (≥7.1)	3 (100)	13 (100)	8 (100)	24 (100)	
	Total	3 (100)	13 (100)	8 (100)	24 (100)	

was observed in 88% (44) of SAM children in this study. Vitamin B12 was low in 34% (17) children. Micronutrients can have varied degree of deficiency in SAM children. In this study, cobalt was deficient in all (100%) children tested followed by copper 04 (12%), zinc 05 (9.5%) and molybdenum 03 (12.5%).

In the present study most common presentations of SAM children were URI followed by hepatomegaly, hyperpigmentation, angular cheilitis, tremors, edema, and hypotonia with hyporeflexia. Among the children with tremors, four of them had severe tremors involving tongue. The authors tried to correlate the association between serum levels of vitamin B12 with age, sex and clinical symptoms but could not find the significant association. Though the clinical manifestations of vitamin B12 were well characterized in many case series, the basis for the same remains a question [20].

Anemia was found in 44 (88%) children. Studies by Kumar et al. and Thakur et al. on SAM children showed prevalence of anemia ranging from 81.1% to 88.5% [2, 19] which is comparable to the present findings. The prevalence of vitamin B12 deficiency in present study was 34%. A study from Delhi on 100 anemic children showed vitamin B12 deficiency alone in 14.4%, and combined vitamin B12 and iron deficiency in 22.2% [21]. A larger study from the same place showed vitamin B12 deficiency in 28% of children [22]. Compared to these studies, the present study showed higher prevalence of isolated vitamin B12 deficiency. Chhabra et al., concluded that megaloblastic anemia occurs commonly in malnourished children [23]. The cause for vitamin B12 deficiency in the present study could predominantly be due to dietary habits like delayed introduction of complementary foods, faulty feeding practices and reduced

**Table 4** Status of vitamin B12 and other micronutrients

Nutrient	Valid no.	Median (IQR)	Minimum	Maximum
Vitamin B12 (pg/ml)	50	271.5 (141 – 521)	49	2000
Zinc (µg/dl)	42	143.95 (100.47 – 204.6)	43.96	292.4
Copper (µg/dl)	42	111.65 (83.9 – 142.5)	32.2	208.2
Cobalt (µg/L)	24	0.55 (0.45 – 0.75)	0.29	1.43
Molybdenum (µg/L)	24	1.31 (1.03 – 1.71)	0.46	16.15
Selenium (µg/L)	24	127.48 (98.89 – 144.7)	66.96	182.33
Manganese (µg/L)	24	13.59 (8.93 – 18.22)	7.45	28.36

intake of non-vegetarian foods in both mother as well as delayed introduction of non-vegetarian foods in children.

Interestingly, varied levels of vitamin B12 were noted in the present study. Six (12%) children had high levels of vitamin B12. Similarly high levels of vitamin B12 were seen in the studies by Khalil et al. [24] and Satoskar et al. [25] in protein energy malnutrition (PEM) children. The possible mechanism quoted by them was probably due to the inflammation in liver causing inadequate storage.

Micronutrient levels including zinc, copper, molybdenum, selenium, manganese and cobalt were also analyzed in these SAM children. Cobalt was the most common deficient micronutrient followed by copper, zinc and molybdenum, whereas levels of selenium and manganese were normal. Cobalt was low in all 24 tested children. Probably the present study is the first study to report this high number of cobalt deficiency in severe acute malnutrition. Many publications have highlighted on cobalt toxicity [26]. Cobalt is an essential element and integral part of vitamin B12, hence it is needed for erythropoiesis and for maintaining nervous system. There is no clear recommended daily allowance (RDA), because it is recommended for vitamin B12 deficiency.

There are not many studies regarding copper and molybdenum levels in children [27]. Studies on adults and pregnant women reported copper deficiency to be around 29 to 34 percent [28]. Only 12% and 13% of present children had copper and molybdenum deficiency respectively. Copper is an essential component of many redox enzymes (cytochrome oxidase). Molybdenum is a cofactor for several breakdown enzymes. Both help in maintaining and promoting strong and healthy bones and nervous system.

In the present study, zinc deficiency was less as compared to other studies [29]. Limited epidemiological studies showed high prevalence of zinc deficiency. Kapil et al. observed that in five major Indian states, overall zinc deficiency was 43.8% among children aged 6–60 mo [29]. However a study from Punjab in children aged 6 mo to 5 y reported only 18% of zinc deficiency [30]. Low prevalence of zinc deficiency could be because some children might have possibly, received zinc either as supplementation or as therapy (for diarrhea/pneumonia) prior to admission. Based on the above findings, authors postulate that in all SAM children many micronutrient deficiencies exist but their prevalence varies.

The strength of present study is the combined assessment of serum levels of vit. B12 along with other common micronutrients, whose deficiencies are very prevalent in SAM children. However, the present study is limited by small sample size and some of micronutrients were not estimated in all children. To conclude, vitamin B12 deficiency is common in SAM children especially in 7–12 mo age group. Vitamin B12 levels can even be high in some SAM children. Varied prevalence of micronutrient deficiencies was observed with

cobalt, copper, zinc and molybdenum and vitamin B12 deficiency. Prevalence of low vitamin B12 and cobalt were more common than deficiency of other micronutrients.

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**Authors' Contributions** LK, VHR: Concept and designed the study, analysed data and drafted manuscript; ASK: Helped in collecting the case, analysed and interpreted the data; SRF, RHD: Helped in collecting the cases, supervised at site, analysed and interpreted the data. All authors reviewed the systematic review and revised the manuscript. All authors have read and approved the final manuscript. VHR will act as guarantor for this manuscript.

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## Declarations

**Conflict of Interest** None.

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