

The prevalence of serum B12 deficiency in infants and the correlation between infant’s and mother’s vitamin B12 levels

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Abstract

Background: Vitamin B12 deficiency is highly prevalent among infants in India. That too may be as a result of low vitamin B12 level mothers and infants are prone to develop its long-term side effects like neurodevelopmental delay.

Objective: To study the prevalence of serum B12 deficiency in infants with clinical features suggestive of vitamin B12 deficiency and to find out the correlation between serum vitamin B12 level of infant’s and mothers.

Method: This prospective observational study included 95 infants and their mothers. Infants who are having symptoms suggestive of vitaminB12 deficiency and their mother’s serum sample were collected to assess their vitamin B12 level. Data was recorded on a pre-designed proforma.

Results: High prevalence of Vitamin B12 deficiency was found in infants and their mothers and their levels correlated significantly with each other (p=0.00). Major signs noticed in children with vitamin B12 deficiency

was hyperpigmentation followed by tremor and glossitis. Mean vitamin B12 level was low in those who belonged to low socio-economic status. Infants of mothers having vegetarian food habits were having more prevalence of vitamin B12 deficiency. Infant’s lower weight was positively associated with vitamin B12 deficiency. Hyperpigmentation was the symptom with maximum negative predictive value and highest sensitivity other than anemia.

Conclusion: High prevalence of Vitamin B12 deficiency was found among infants and their mothers. There is a positive correlation between infant and maternal vitamin B12 levels. Maternal dietary habits also have an influence on infant’s vitamin B12 level.

Keyword: Vitamin B12 level, Mother, infant, hyperpigmentation, early child development, nutritional deficiency

Introduction

Vitamin B12 is a water-soluble micronutrient that plays various roles in the body. It serves as a coenzyme for cytosolic methionine synthase (MS) and mitochondrial

methyl malonyl-CoA mutase (MCM).¹ Vitamin B12 (cobalamin) (B12) is essential for the development of the fetus and child. Human species does not synthesize B12 and therefore, the exogenous intake is essential. In early life infants get vitamin B12 exclusively from mother's milk.^{2,3}

Common dietary sources include meat, fish and dairy products that have been fermented. After exposure to pancreatic proteases, vitamin B12 is released from the R proteins in the small intestine and forms a complex with intrinsic factor. The intrinsic factor–vitamin B12 complex is taken up in the terminal ileum. Then the complex dissociates and enters the portal circulation bound to transcobalamin II, which transports vitamin B12 to tissues.⁴

The primary clinical manifestations of vitamin B12 deficiency in children and adolescents includes general weakness, fatigue, anorexia, failure to thrive, irritability, developmental delay/regression, paresthesia's, impaired vibratory and proprioceptive sense, hypotonia, seizures, ataxia, dementia, paralysis, abnormal movements, memory loss, personality change, poor school performance, depression.⁵

Deficiency of vitamin B12 can adversely affects the neurological development of the infants also. Infants may be subjected to such adverse effects probably because of the low vitamin B12 level of mother. This in turn is reflective of the widely prevalent vegetarian food habits of people. The non-vegetarians in India too consume very little of non-vegetarian food per week, not enough to mitigate the effects.⁶

During pregnancy, vitamin B12 is concentrated in the fetus and stored in the liver. Infants born to vitamin B12-replete mothers have stored of vitamin B12 that are adequate to sustain them for the first several months

postpartum. Vitamin B12 deficiency rarely occurs before about 4 months of age. Infants of vitamin B12-deficient breastfeeding mothers, or infants receiving low amounts of animal-source foods, may be vulnerable to vitamin B12 deficiency between 6 and 12 months of age.⁷

Deficiency of cobalamin, whether nutritional or due to inborn errors of cobalamin metabolism, inactivate MS and MCM leading to the accumulation of homocysteine (Hcy) and methyl malonic acid (MMA), respectively. In conjunction with total B12 and its bioactive protein-bound form, holo-trans cobalamin (holo-TC), Hcy, and MMA are the preferred serum biomarkers utilized to determine B12 status.⁸

Materials & methods

Current study is a cross sectional prospective study conducted between December 2019 and September 2021 after getting approval from the institute approval committee. The aim of this study was to study the prevalence of serum B12 deficiency in infants with clinical features suggestive of vitamin B12 deficiency and to find out the correlation between serum vitamin B12 of infants and their mothers. As per the calculated sample size 95 infants with anemia and with clinical features suggestive of vitamin B12 deficiency like hyper pigmentation, seizures, glossitis, hypotonia, developmental delay, chorea, tremors and macrocytotic/megaloblastic picture in the hematological examination, and their mothers were included in the study.

Sample size

Total 95 infants with their mothers. Sample size calculated as follows: The prevalence of vitamin B12 deficiency in infants reported as 57%. Using the sample size formula for the prevalence,

$$n = (z_{\alpha/2})^2 pq/d^2$$

At 5% level of significance $z_{\alpha/2} = 1.96$ and taking the anticipated absolute difference as 10%

$$n = [(1.96)^2 \times 57 \times 43] \div (10)^2 \\ = 94.15$$

Infants with h/o perinatal insult, infants with cerebral palsy, infants with meningitis, infants with h/o blood transfusion, infants with h/o GI surgeries were excluded from the study.

Proforma included data regarding demographic variables of mother, maternal characteristics (age, parity, dietary habits, occupation), infant characteristics (dietary habits, feeding difficulty) anthropometric variables of infant (weight, height, weight/height and head circumference) and vitamin B12 supplementation, clinical features of vitamin B12 deficiency (seizure, hyperpigmentation, tremor, hypotonia, developmental delay, glossitis, eye movement abnormalities) and hematological parameters of infants. Developmental delay was assessed with the help of TDSC.⁹

Samples were collected in non-EDTA vials and immediately transported on ice to the laboratory. Samples were centrifuged and stored at -70°C for assessment of vitamin B12

level. Chemiluminescent immunoassay (CLIA) was done to assess the level.

Statistical analysis

Data was statistically analyzed using the SPSS (statistical package for social sciences, Chicago, IL, USA) software version 16.0 and MS EXCEL. The Chi-square test was used to compare the qualitative data. Pearson coefficient was used to find the correlation between maternal and

infant's Vitamin B12 level. p value <0.05 was considered significant.

Results

In this study vitamin B12 level was found to be inadequate in 72.63% of infants with symptoms suggestive of vitamin B12 deficiency and in 50.52% of the mothers. So high prevalence of Vitamin B12 deficiency was found among infants and mothers. Their serum vitamin B12 levels were significantly correlated with each other ($p=0.00$, $r=0.745$). (Table:2), (figure:1).

On univariate analysis, none of the socio demographic factor was significantly associated with vitamin B12 deficiency except the dietary habits of mother. Among infants residing in urban areas 42.3% were deficient, whereas 53.6% infants were deficient from rural population. Those born in the lower socio-economic status families (as per the Kuppuswami scale) were deficient to the extent of 75.8%, whereas 66.7% of lower middle class were deficient and the mean was also higher in later category ($m=448.22$) compared to the former ($m=207.4$). Though these are not statistically significant, they do reflect the effect of residence and socio-economic status on the vitamin B12 levels of infants. The mean vitamin B12 level among infants with their mothers being vegetarian was 199.03, and that among infants of non-vegetarian mothers was 473.36. There was also a positive correlation with the diet of mother and the vitamin B12 level in infants ($p=0.004$). Among non-vegetarian mothers' frequency of non-vegetarian food also had a positive correlation with infant vitamin B12 level ($p=0.00$).

Table 1: Relation of Maternal Characteristics with infant Vitamin B12 level.

Sn.	Variables	Vit B12 deficiency (≤300pg/ml)	Vit B12 adequacy (>300pg/ml)	Chi square p value	Mean
1)	Residence ▪Rural ▪Urban	53(76.8%) 16(61.5%)	16(23.2%) 10(38.5%)	0.137	226.58 292.18
2)	Socioeconomic status ▪ Lower middle ▪Upper lower ▪Lower	4(66.7%) 49(71%) 16(80.6%)	2(33.3%) 20(29%) 4(20%)	0.689	448.22 244.83 207.41
3)	Religion ▪Hindu ▪Muslim	67(73.6%) 2(50%)	24(26.4%) 2(50%)	0.300	234.90 463.7
4)	Age ▪20-30yrs ▪30-40yrs	63(72.4%) 06(75.0%)	24(27.6%) 02(25.0%)	0.875	248.03 206.58
5)	Education ▪Illiterate ▪Literate - <10th class - ≥10th class	4(75.0%) 40(50.0%) 4(40.0%)	1(25.0%) 40(50.0%) 6(60.0%)	0.367	175.13 252.80 257.52
6)	Dietary habit ▪Vegetarian ▪Eggetarian ▪Non-vegetarian	55(78.5%) 9(60.0%) 5(50.0%)	15(31.4%) 6(40.0%) 5(50.0%)	0.004	199.03 304.36 473.36

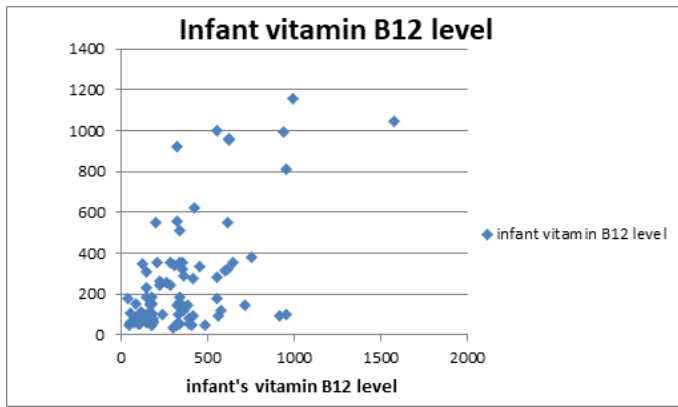
Table 2: Correlation of Maternal and infants Vitamin B12 level

	Pearson correlation coefficient	p value
Maternal vitamin B12 & newborn's vitamin B12	0.610**	0.000(<0.01)

** . Correlation is significant at the 0.01 level (2-tailed).

P value < 0.001 highly significant; < 0.05 significant; > 0.05 not significant

Figure 1: Linear regression curve showing correlation of infants and maternal vitamin B12 level.



Among infants with vitamin B12 deficiency hyperpigmentation was the most common symptom identified (72.6%), in which 43.47% had only knuckle hyper pigmentation, 31.88% had hyper pigmentation over knuckle and dorsum of hands or feet and 18.88% had hyper pigmentation over thighs or trunk also. Tremor was found in 26% (n=18) of the infants, developmental delay in 33.33% (n=23), glossitis in 20.28% (n=14) and seizure was seen in only 1 infant with vitamin B12 deficiency. Other features like eye movement abnormalities and chorea were not seen in any of the infant with vitamin B12 deficiency. All infants in the study were anemic. (Table:3)

All the infants included in the study were having anemia as it was an inclusion criterion. Other than anemia the symptom which had highest sensitivity was hyperpigmentation (94.20%). The one with highest specificity as well as positive predictive value was tremors (100%). Single symptom which had maximum negative predictive value was hyper pigmentation (76.47%). Among all the symptoms the one which had highest overall accuracy was also hyperpigmentation. (Table:4)

Among the infant's anthropometric variables weight was significantly associated with vitamin B12 level(p=0.001). Other variables like head circumference or length had no significant relation with vitamin B12 level.

Table 3: Relation of clinical characteristics of B12 deficiency in infants and vitamin B12 level

Sn.	Variables	Vit B12 deficiency	Vit B12 Adequacy	P value
1)	Hyper pigmentation	65(83.3%)	13(16.7%)	0.00
	▪knuckles only		10(25%)	
	▪knuckle+ dorsum of hand and foot	30(75%)	3(12%)	
	▪knuckle+ dorsum of hand and foot+ thighs	22(88%)	0(0.0%)	
	▪no hyperpigmentation	13(100%)	13(76.5%)	
		4(23.5%)		
2)	Tremor			0.004
	▪Present	18(100%)	0(0.0%)	
	▪Absent	51(66.2%)	26(33.8%)	
3)	Pallor			-
	▪Present	69 (72.6%)	26(27.4%)	
	▪Absent	0(0.0%)	0(0.0%)	
4)	Seizures			0.007
	Present	1(20.0%)	4(80%)	
	Absent	68(75.6%)	22(24.4%)	
5)	Developmental delay			0.333
	Present	22(75.8%)	7(24.1%)	
	Absent	46(69.7%)	20(30.3%)	
6)	Hypotonia			0.101
	Present	0(0.0%)	1(100%)	
	Absent	69(73.4%)	25(26.6%)	
7)	Glossitis			0.487
	Present	14(66.7%)	7(33.3%)	
	Absent	55(74.3%)	19(25.7%)	

Table 4: Predictability of Symptoms in Vitamin B12 Deficiency in Infants

	Tremor	Hyper pigmentation	Developmental delay
Sensitivity	26.09%	94.20%	33.33%
Specificity	100%	50.0%	76.92%
Positive	100%	83.33%	79.31%

Predictive value			
Negative Predictive value	33.77%	76.47%	30.30%
Positive Likelihood ratio	-	1.88	1.44
Negative Likelihood ratio	0.74	0.12	0.87
Overall accuracy	46.32%	82.11%	45.28%

Discussion

In present study it is found that there is high prevalence of Vitamin B12 deficiency in infant's (72.6%) with features suggestive vitaminB12 deficiency in the Central India. There is a high prevalence among their mothers also (50.52%) with a positive correlation among their vitamin B12 levels. Similarly, the studies done in different parts of India in past showed high prevalence of Vitamin B12 deficiency in infants and children of various ages and in their mothers. Medha Mittal, et al in a study conducted at tertiary care hospitals in North India in infants 1 to 6 months of age found an alarming rate of vitamin B12 deficiency in young Indian infants (57%) and their mothers (46%). They also got a positive correlation ($r = .23$) between the B12 levels of the infants and their mothers.⁶ Julia L. Finkelstein et al, in a study conducted in Karnataka, India, also found out a significant association between maternal vitamin b12 status during pregnancy with infant vitamin B12 status. This study was one of the largest prospective study to date to examine the burden of vitamin B12 status in pregnancy and its association between neonatal B12 status and perinatal outcomes.¹⁰ In studies conducted outside India like in Turkey, Nepal, Guatemala also have

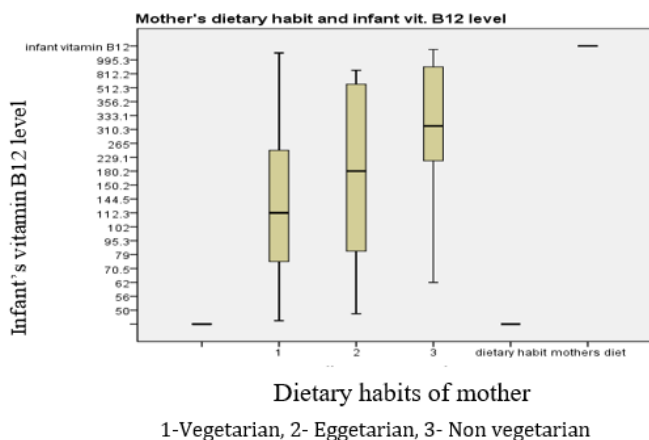
similar correlations. In a study conducted by Ahmet Koc et al, Sanliurfa Maternity Hospital, Turkey found out a statistically significant correlation between maternal and cord blood serum vitamin B12 levels ($r = 0.395$, $P < 0.001$).¹¹ So all these shows that Vitamin B12 status of mother needs to be improved which will eventually improve the VitaminB12 status of infant.

In our study mean vitamin B12 level in infants who are exclusively breastfed was 211.9pg/L which was lower than who were given breast milk and supplementary feed($M=220.84pg/L$), which was much lower than those got supplementary feed only(892.45pg/L). Similarly, in a study conducted in New Delhi by Sunita Taneja et al, found that Vitamin B12 concentration in 6–11-months old children was significantly lower in breastfed (183; 120–263 pmol/L) than in non-breastfed (334; 235–463pmol/L) children.¹² In our study among Infants with more than 6 months of age and who were exclusively breastfed, 47.2% were having vitamin B12 deficiency. This was another important finding in our study which indicates that timely initiation of complimentary feeds is still lacking in spite of so much of awareness programmes and that is leading to vitamin B12 deficiency in infants also.

In the current study infants born to mothers who are vegetarian are having low mean vitamin B12 level (199.03pg/ ml) as compared to non-vegetarian group (473.36pg/ ml), the observation was statistically significant also ($p=0.004$). (Table:1) (Figure:2). Anna M Christian et al in a study conducted in Mysore, India, showed consumption frequencies of non-vegetarian foods were positively associated with plasma B12 concentrations ($P<0.01$).¹³ This shows the importance of vitamin B12 supplementation for pregnant and lactating women having predominantly vegetarian food habits and those

belongs to low socio-economic status who are not even getting enough vegetarian sources of vitamin B12 either. None of the mothers in the current study had got vitamin B12 supplementation, even during pregnancy.

Figure 2: Box and Whisker plot showing the relation between mothers dietary habits and infant's vitamin B12 level



Christopher Duggan et al, conducted a case control prospective study in in Bangalore, India in a case group of pregnant women who were given vitamin B12 during pregnancy and a placebo group. At 6 wk postpartum median breast milk vitamin B-12 concentration was 136 pmol/L in vitamin B-12-supplemented women vs. 87 pmol/L in the placebo group (P < 0.0005). Vitamin B12 level of infants at 6 months was a significantly high in control group compared to placebo group(p=0.001).¹⁴

This will also help to prevent various complications of vitamin B12 deficiency in infants including neuro developmental delay. In our study 33.33% children with vitamin B12 deficiency had developmental delay. In a study conducted by Selahattin Katar et al in 9 to 36 months old children, could find hypoactivity, decreased attention, lethargy, and unhappy facial expression, and neuromotor developmental delay in all the children who were having vitamin B12 deficiency. They also got a statistically significant relationship between serum

vitamin B12 levels of the mothers of these children (P= 0.001).¹⁵

Among the symptoms the one with highest specificity as well as positive predictive value was tremors (100%). Hyperpigmentation had maximum negative predictive value (76.47%) and highest sensitivity (94.20%) after anemia and was also the symptom which has highest overall accuracy. This information is helpful in the diagnosis vitamin B12 deficiency in a primary set up where such tests are not available or if the parents are not affordable.

Conclusion

Child brain grows moment by moment, as it interacts with environment in optimum nutritional milieu and other factors. In the first few years of life, more than one million neural connections are formed each second- a pace never repeated again. The quality of a child's early experiences makes a critical difference as their brain develop, providing either strong or weak foundations for learning, health and behavior throughout life. The highly prevalent Vitamin B12 deficiency among both mother and infants needs to be addressed urgently as it may adversely influence Early Child Development (ECD).

Highly significant positive correlation between infant and mother serum B12 level and significant correlation between maternal dietary habits and infant B12 level should be utilized to improve infant B12 level.

Health care providers needs to be sensitized for identifying signs of Vitamin B12 deficiency in infants like anemia, hyperpigmentation, tremor and development delay. Early treatment would definitely curtail the effect of B12 deficiency on Early Childhood development.

WHAT IS ALREADY KNOWN?

There is high prevalence of Vitamin B12 deficiency in India.

WHAT THIS STUDY ADDS?

Maternal Vitamin B12 status and dietary habits has a significant impact on infant vitamin B12 level.

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