

ORIGINAL RESEARCH

To study the relationship between Vitamin B12 deficiency in breast-fed infants and their mothers and its clinico hematological manifestations: A cross sectional study

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ABSTRACT

Background: Vitamin B12 is essential for nerve and blood cell proper functioning and helps make DNA, the genetic material of the cell. In Indian infants, its deficiency is prevalent but there is limited data available on it. Its early recognition and prompt treatment is necessary to avoid permanent neurological damage. **Objectives:** To study the relationship between Vitamin B12 deficiency in breast-fed infants and their mothers and its clinico-hematological manifestations. **Methods:** A case control study was conducted in Department of Pediatrics, Career institute of medical sciences, Lucknow from March 2023 to August 2023 including children between 1-12 months of age and consuming breast milk in their diet. After prior informed consent, all the eligible infants who were hospitalized in the Department of Pediatrics were enrolled for the study. A detailed history and examination was carried out. Malnutrition if present was classified as per WHO classification using the Z scores. Complete blood count and Vitamin B12 level of all enrolled infants and their mothers was done. Vitamin B12 Deficiency would be Defined as: Plasma Vitamin B12 level <200pg/ml (150pmol/L). **Results:** Among total of 319 infants, 230 infants were anemic and out of them 62 infants (27%) were Vitamin B12 deficient, the relationship between anemia in infant and their vitamin B12 level was statistically significant (p=0.001). Overall the prevalence of vitamin B12 deficiency in study population was found to be 21.3%, the difference between the prevalence of wasting and stunting in Vitamin B12 deficient infants vs non deficient infants was statistically not significant. Of 319 mothers, 72(22.6%) were Vitamin B12 deficient and among them 58 (80.6%) had infants who were also deficient (p<0.001). The odd's of having Vitamin B12 deficient infant if the mother is Vitamin B12 deficient is 98.19 (95% CI 41.5-232.3)

Keywords: Maternal Vitamin B12, infant, mother's milk, anemia, vitamin B12 deficiency

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BACKGROUND

Vitamin B12 is a water soluble vitamin found mostly in foods of animal origin. As a significant proportion of Indian population is vegetarian and moreover Vitamin B12 is also not supplemented routinely as a part of the 'National Iron plus Initiative', so children as well as their mothers are at risk of developing Vitamin B12 deficiency.

Vitamin B12 is essential for nerve and blood cell proper functioning and helps make DNA, the genetic material of the cell. Vitamin B 12 deficiency leads to megaloblastic anemia, poor memory, abnormal neurological signs and symptoms and neurological regression.¹ Mother's milk is a boon for infant during early stages of life even when applied orally.²

Moreover, in india women choose to deliver at home, which further deprives them of proper health care counselling regarding proper nutrition, breastfeeding and baby care.³

However, there is limited data available on the effect of Vitamin B12 deficiency in a lactating mother on her infant's Vitamin B12 level, especially in India. So, this study was planned to overcome these knowledge gaps.

METHODS

A case control study was conducted in Department of Pediatrics, Career institute of medical sciences, Lucknow from March 2023 to August 2023 including children between 1-12 months of age and consuming

breast milk in their diet. Infants not on breast milk or had blood transfusion in last 4 weeks or have taken Vitamin B12 supplements in last weeks were excluded from study

After prior informed consent, all the eligible infants who were hospitalized in the Department of Pediatrics were enrolled for the study. A detailed history and examination was carried out including details about breast feeding, complimentary feeding, intake of bovine milk etc. Detailed anthropometric examination of the infant and mother was performed. Malnutrition if present was classified as per WHO classification using the Z scores⁴

Complete blood count and Vitamin B12 level of all enrolled infants and their mothers was performed.

Test for measuring Vitamin B12 levels involved the ARCHITECT B12 assay method which is based on the Chemiluminescent Microparticle Immunoassay (CMIA) technology. Vitamin B12 Deficiency would be Defined as: Plasma Vitamin B12 level <200pg/ml (150pmol/L).^{5,6}

STATISTICAL CONSIDERATIONS

SAMPLE SIZE

With an anticipated prevalence of 29% as per Houghton LA et al⁷, precision of 95%, 95% confidence, sample size calculated is 317

$$n = Z^2 pq/d^2$$

n- Desired sample size

Z=standard normal deviate; usually set at 1.96 (or a-2), which correspond to 95% confidence level.

P=proportion in the target population estimated to have a particular characteristics.

Q=1-p (proportion in the target population not having the particular characteristics)

D=degree of accuracy required, usually set at 0.05 level (occasionally at 2.0)

STATISTICAL ANALYSIS

The data collected were tabulated using Microsoft Excel software and statistically evaluated using SPSS v 16.0 version for Windows. The results were presented as mean \pm SD and percentages. The Chi-square test was used to compare the categorical variables/dichotomous variables. The Kolmogorov-Smirnov test for normality was used to check whether the data were normally distributed or not. The independent-sample t-test was used to compare the means of the parametric data and the Mann Whitney U test was used to compare the mean scores between the non-parametric data. A p-value<0.05 was considered significant.

OBSERVATION AND RESULTS

During the study period of 6 months, total number of patients who were included in this study were 319. Baseline characteristics has been shown in **Table 1**

HEMATOLOGICAL CHARACTERISTICS OF STUDY POPULATION (Table 2)

In the study population of infants the mean hemoglobin was 9.81g / dl (SD=2.57). The maximum Hb was 21.3gm / dl and minimum Hb was 2.4gm/dl. Haematological profile is given in table 2

Among total of 319 infants, 230 infants were anemic and out of them 62 infants (27%) were Vitamin B12 deficient while 168/230 were normal vitamin B12 level, 89 infants were non anemic and out of them 6 infants (6.7%) were Vitamin B12 deficient and 83/89 were normal vitamin B12 level. So the relationship between anemia in infant and vitamin B12 level was statistically significant (p=0.001).

Out of 319 infants, 68(21.3%) infants had Vitamin B12 deficiency in our study population. The prevalence of wasting and stunting in Vitamin B12 deficient infants was 64.7% and 55.9% respectively while in infants having normal vitamin B12 the prevalence of wasting is 49% and prevalence of stunting is 44.6%. The difference was statistically not significant.

Among the Vitamin B12 deficient infants, 7 (10.2%) had clinical features of Infantile tremor syndrome. All these 7 infants had developmental regression associated with tremors. The clinical profile of these 7 patients was as depicted in the table 3

Among the Vit. B12 deficient infants, mean MCV value was 108.90 while in the normal Vitamin B12 level group mean MCV was 87.70. This difference in mean MVC was statistically significant (p<0.001) as described in table 4

Effect of maternal vitamin B12 level on infants vitamin B12 level:

In the study population of mothers, out of 319 mothers, 72(22.6%) were Vitamin B12 deficient and 247(77.4%) had normal Vitamin B12 levels. Among the 72 deficient mothers, 58 (80.6%) had infants who were also deficient. Whereas, among the mothers with no Vitamin B12 deficiency (n=247), only 4% (n=10) had deficient infants. This relation between Vitamin B12 deficiency in mothers and vitamin B12 deficiency in their infants is statistically significant (p<0.001). The odd's of having Vitamin B12 deficient infant if the mother is Vitamin B12 deficient is 98.19 (95% CI 41.5-232.3) as shown in table 5

Effect of maternal anemia on infants vitamin B12 level(table 6):

In the study population of mothers, out of 319 mothers, 267(83.7%) were anemic and 52(16.3%) were non anemic. Among the 267anemic mothers, 64 (24%) had infants who were vitamin B12 deficient. Whereas, among the 52 non anemic mothers, only 7.7% (n=4) had vitamin B12 deficient infants. This relation between Anemia in mothers and vitamin B12 deficiency in their infants is statistically significant (p<0.001). The odd's of having vitamin B12 deficient

infant if the mother is anemic is 3.78 (95% CI 1.3-10.9)

Effect of maternal anemia on infant’s anemic status (table 7):

In the study population of mothers, out of 319 mothen, 267(83.7%) were anemic and 52(16.3%) were non

anemic. Among the 267 anemic mothers, 197(73.8%) had infants who were also anemic. Whereas, among the 52 non anemic mothers, 63.5% (n=33) had anemic infants. This relation between Anemia in mothers and anemia in their infants is statistically not significant (p=0.129). The odds of having anemic infant if the mother is anemic is 1.62 (CI 0.87 - 3.03)

Table 1: Baseline characteristics of neonates

	No of infants	Percent
Term born	255	79.9%
Pre term born	64	20.1%
Exclusive breastfed (<6 month)(n=177)	153	86.4%
Taking bovine milk (>6month)(n=142)	93	65.5%
Taking solid food (>6month)(n=142)	112	78.8%
H/O past hospitalisation(n=319)	253	79.3%

Table: 2 HEMATOLOGICAL CHARACTERISTICS OF STUDY POPULATION

Infant’s Parameter	Mean	SD
HEMOGLOBIN (g/dl)	9.81	2.57
MCV (fl)	92.22	11.73
MCH (pg)	28.13	4.05
MCHC(g/dl)	32.91	4.12
RDW (%)	18.71	3.40
S. VITAMIN B12(pg/ml)	532.06	420.46

Table 3: Clinical manifestation of vitamin B12 deficiency in infants

Patient number	Age (months)	Wt for length Z score	Length for age Z score	Hb (mg/dl)	MCV (fL)	Vit B12 level (pg/dl)	Clinical features
Patient 1	11	-1to-2SD	0to-1SD	6.6	112	90	Tremor Developmental delay
Patient 2	8	-1to-2SD	0to-1SD	12	102	88	Tremor Developmental delay
Patient 3	4	<-3SD	<-3SD	8	107.2	176	Tremor Developmental delay
Patient 4	10	-2to-3SD	0to-1SD	5	100	80	Tremor Developmental delay
Patient 5	5	-1to-2SD	-1to-2SD	9	98	164	Tremor Developmental delay
Patient 6	7	-2to-3SD	<-3SD	6.8	104	210	Tremor Developmental delay
Patient 7	9	-1to-2SD	-2to-3SD	7.2	101	148	Tremor Developmental delay

Table 4: Comparison of RBC indices in infants with Vitamin B12 deficiency with those with normal Vitamin B12

Vit. B12 of Infants		N	Mean	Std. Deviation	p-value
MCV(fL)	Deficient	68	108.90	5.10657	<0.001
	Normal	251	87.70	8.46977	
RDW (%)	Deficient	68	19.57	3.34902	0.018
	Normal	251	18.48	3.38735	
Hct (%)	Deficient	68	28.10	8.87604	0.027
	Normal	251	30.56	7.90545	

Table 5: Effect of maternal vitamin B12 level on infants vitamin B12 level

		Vitamin B12 of Infants		Total
		Deficient	Normal	
Vitamin B12 of Mothers	Deficient	58 80.6%	14 19.4%	72 100.0%
	Normal	10 4.0%	237 96.0%	247 100.0%
Total		68 21.3%	251 78.7%	319 100.0%

Applied χ^2 test for significant. χ^2 value=194.6; p-value=<0.001;

Odd's Ratio=98.19 (95% CI 41.5-232.3); consider significant.

Table 6: Effect of maternal anemia on infants vitamin B12 level

		Vitamin B12 of Infants		Total
		Deficient	Normal	
Anemia (Mothers)	Anemic	64 24.0%	203 76.0%	267 100.0%
	Non-Anemic	4 7.7%	48 92.3%	52 100.0%
Total		68 21.3%	251 78.7%	319 100.0%

Applied χ^2 test for significant. χ^2 value=6.87; p-value<0.001; Odd's ratio=3.78 (95% CI 1.3 - 10.9);consider significant.

Table 7: Effect of maternal anemia on infant's anemic status

		Anemia of Infants		Total
		Anemic	Non-Anemic	
Anemia (Mothers)	Anemic	197 73.8%	70 26.2%	267 100.0%
	Non-Anemic	33 63.5%	19 36.5%	52 100.0%
Total		230 72.1%	89 27.9%	319 100.0%

Applied χ^2 test for significant. χ^2 value=2.305; p-value=0.129; consider insignificant.

DISCUSSION

This study intended to find out the relationship between Vitamin B12 deficiency in breast-fed infants and their mothers and to study the clinical and hematological manifestations of Vitamin B12 deficiency in infants

PREVALENCE OF VITAMIN B12 DEFICIENCY

In this study, 21.3% of infants were suffering from vitamin B12 deficiency and 22.6% lactating mothers were suffering from vitamin B12 deficiency. This is in contrast to Jones M. Katharine et al⁸ who reported that 2/3rd of women (80% of whom were lactating) and half of their infants had low or marginal status at 12 months postpartum.

There is very few data on the prevalence of cobalamin deficiency in Indian infants and children, and to the best of our knowledge, no data are available for the 1-12 months age group.

In this study we found anemia in 72.1% of infants and 21.3% infants were deficient in vitamin B12, 21% infant had macrocytic anemia. This is similar to Honzík Tomas et al ,2010⁹ reported that 63% of breastfed infants of children had anemia (megaloblastic in 28% of all children).

Out of 319 infants, 68(21.3%) infants had Vitamin B12 deficiency in our study population. The prevalence of wasting and stunting in Vitamin B12 deficient infants was 64.7% and 55.9% respectively while in infants having normal vitamin B12 the prevalence of wasting is 49% and prevalence of stunting is 44.6%. The difference was statistically not significant.

This is much higher as compared to Akcaboy M et al¹⁰ where a total of 20 infants with a mean age of 6.65 ± 4.5 mo were included in the study. The weight and height were below the third percentile only in four patients.

Of 319 mothers, 72(22.6%) were Vitamin B12 deficient and among them 58 (80.6%) had infants who were also deficient (p<0.001). The odd's of having Vitamin B12 deficient infant if the mother is Vitamin B12 deficient is 98.19 (95% CI 41.5-232.3). This is in

contrast to to Akcaboy M et al¹⁰ where all of the mothers had vitamin B12 deficiency.

Our results are similar to Mittal et al¹¹ who found that the prevalence of B12 deficiency in infants was 57% and 46% of mothers were deficient. There was a positive correlation (r = .23) between the B12 levels of the infants and their mothers.

INFANTILE TREMOR SYNDROME

In this study 10.2% of vitamin B12 deficient infants were having Infantile tremor syndrome.(ITS) is characterized by anemia, skin depigmentation, hyperpigmentation at knuckles, tremors, and developmental delay. Gowda VK et al^{12,13} reported that ITS should be considered in children 3 years with anemia, developmental delay/regression, and skin depigmentation, with/without tremors. ITS can be seen in <3 months of age and in high socio-economic status. In their study, they also found the developmental delay was in 90%, regression in 8.5%, and tremors in 57%.

STRENGTHS OF THE STUDY

A total of 319 infants (mean age 6.16 months, M:F 1.68:1) and their mothers were enrolled in the study, which was as per our sample size calculation with a precision of 95% and 95% confidence. Very few studies have been done on prevalence of vitamin B12 deficiency in infants of age group 1 month - 12 month and their lactating mothers. We included infants and their lactating mothers. This allowed us to know the relationship between the status of infant's vitamin B12 and the mother's vitamin B12 status.

LIMITATIONS OF THE STUDY

The prevalence of anemia in both infant as well as the mother population was very high, which could have been due to iron deficiency. Estimation of serum ferritin and serum folate levels may have helped us establishing the other causes of nutritional anemia. These investigations could not be performed due to financial limitations.

CONCLUSION

The results of present study reveal that there is a high prevalence of anemia in our cohort of infants as well as their mothers. Vitamin B12 deficiency was responsible for anemia in about a quarter of these cases. As Vitamin B12 deficiency can cause significant neurological morbidities especially in infants, so prevention is of utmost importance in this susceptible age group. From this study we also gathered evidence to prove that maternal B12 deficiency is very strongly associated with deficiency in infants. So, in order to improve Vitamin B12 status of infants, we need to focus our attention in improving the Vitamin B12 intake of their mothers. This can be achieved by dietary counselling, spreading awareness about Vitamin B12 rich food items and by food fortification. There may even be a need to supplement Vitamin B12 along with the routine IFA (Iron Folic Acid) supplementation being given to pregnant women. However, further studies are warranted to provide evidence regarding the feasibility and success of this supplementation.

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