

ORIGINAL ARTICLE

Prevalence of Vitamin B12 Deficiency among Exclusively Breast Fed Term Infants in South India

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ABSTRACT

Objective: Vitamin B12 is not synthesized in the body and its only dietary sources are non-vegetarian. The breast milk of mothers in resource poor countries who are on predominantly vegetarian diets is deficient in vitamin B12. Hence exclusive breast feeding (EBF) may result in B12 deficiency in the infant, which can affect the neurodevelopmental outcome. Our aim was to study the serum vitamin B12 levels among EBF infants and identify the risk factors for B12 deficiency.

Method: This cross-sectional study was done among EBF, term, otherwise healthy infants, 1–6 month of age in the well-baby clinic. The sociodemographic data of mother and the infants' anthropometric measurements were noted and blood samples were sent for complete blood count and serum vitamin B12 levels. The data were analysed using SPSS software version 16.

Results: We enrolled 149 EBF infants, aged 1–6 months and the mean age was 3.1 (± 1.03) months. The mean serum vitamin B12 level was 199.91 (± 112.523) pg/ml. Low serum vitamin B12 levels (<200 pg/ml) was seen in 95 (63.7%) infants. On multivariate analysis, there were no other significant risk factors for B12 deficiency in the infants.

Conclusion: The prevalence of vitamin B12 deficiency among EBF infants is 63.7%. Because of its importance in neurological development during infancy, there is an urgent need to address this issue while promoting exclusive breast feeding.

INTRODUCTION

Vitamin B12 not synthesized in the body and its dietary sources are non-vegetarian. B12 deficiency in infancy has been reported to cause delay/regression of neurological development. If detected early, the

delay may be reversed but the long-term prognosis is not clear [1]. World-wide, exclusive breast feeding is being vigorously promoted in view of its tremendous benefits. However, in low to middle income countries the milk of mothers may be deficient in vitamin

B12 [2, 3]. If their infants are exclusively breast feed (EBF), they may develop B12 deficiency [4]. Studies from India report varying prevalence of B12 deficiency in different age groups. Among adults in North India, a prevalence of 47% has been reported [5] while another one reported a prevalence of 57% in infants 1–6 months of age [6].

Since breast milk is the only source of B12 for an exclusively breast fed infant and there are no current guidelines for supplementation of B12 in these infants, this study was done to estimate the serum vitamin B12 levels among EBF infants and identify the risk factors for B 12 deficiency.

METHODS

This cross-sectional study was done with the permission of the Institutional Ethics Committee and the written informed consent of parents, among infants, 1–6 month of age, attending the well-baby clinic of a teaching hospital in South India between August 2018 and January 2020 by convenience sampling. The inclusion criteria were (i) term, appropriate for gestational age, (ii) age 1–6 months and (iii) on exclusive breast feeds. The exclusion criteria were (i) infant or mother on any vitamin supplements other than vitamin D and folic acid, (ii) chronic systemic illness in mother or infant and (iii) suspected developmental delay in the infant as screened by Trivandrum Developmental screening chart. The sociodemographic data and dietary habits of mother with respect to consumption of diary, poultry and meat were noted. Socioeconomic status was classified according to the Modified Kuppusamy's Scale. The infants' anthropometric measurements were done by trained staff and plotted on age and gender appropriate WHO growth charts. Blood was tested for complete blood count by an automated analyser and serum vitamin B12 levels by chemiluminescence method.

Sample size calculation

The reported prevalence of B12 deficiency in India being 57% [7], using N master software, for a precision level of 15% and CI of 95%, the minimum sample size was calculated to be 90.

Data management and analysis

All statistical analysis was performed using Statistical Package for Social Science (SPSS, version 17) for Microsoft windows. Descriptive statistics were presented as numbers and percentages. The data were expressed as Mean and SD. The χ^2 test were used for qualitative data. Multiple logistic regression equation method was used for evaluating the risk factors. A two-sided p value <0.05 was considered statistically significant.

Study definitions

Term infant was defined as an infant born between the end of the 37th week and the end of the 42nd week of gestation (WHO definition).

Appropriate for gestation age was defined as birth weight between 10th and 90th percentiles for gestational age, based on WHO multinational foetal growth charts.

Normal nutrition was defined as weight and length for age and weight for height z scores between -2 and $+2$, WHO growth charts.

Moderate acute malnutrition (MAM) was defined as a weight for length z score between -2 and -3 in the WHO growth charts.

Anaemia: haemoglobin (Hb) < 11 g/dl in both infants and pregnant mothers (WHO definition).

Exclusive breast feeding was defined as taking only breast milk, nothing else (exceptions oral rehydration solution, vitamins, minerals or medicine, WHO).

Low serum vitamin B12 levels < 200 pg/ml [8].

Microcytosis MCV $< 72 \mu\text{m}^3$ /Normocytosis MCV $72\text{--}88 \mu\text{m}^3$ /Macrocytosis $> 88 \mu\text{m}^3$ [9].

Hypochromia MCH < 24 pg/cell/Normochromia MCH: $24\text{--}30$ pg/cell [9].

Dietary pattern definition

The self-reported frequency of animal protein intake by the mothers was noted by interview method. Previous surveys have classified dietary patterns into five major categories [10]. In our study vegans and lacto-ovo-vegetarian were considered vegetarian and the pesco-vegetarian, semi-vegetarian and non-vegetarian as non-vegetarian.

RESULTS

We enrolled 149 infants aged 1–6 month. The mean (SD) of ages of the infants and their mothers, Hb level, serum B12 level, maternal Hb at delivery are depicted in Table 1. Of the 149 infants, 95 (63.7%) had serum vitamin B12 deficiency. Anaemia was present in 57% of the infants and, in 70% of them, it was microcytic hypochromic anaemia. There was no significant relationship between anaemia and serum B12 deficiency. Only five infants had macrocytosis and all of them had B12 deficiency. The total leucocyte count and platelet counts were normal in all the 149 subjects. The clinic sociodemographic profile of the subjects with and without B12 deficiency and the Odd's ratio for each variable are depicted in Table 2. On multivariate analysis, none of these factors were found to be significant risk factors for B12 deficiency in the infants.

DISCUSSION

In our study of 149 EBF infants, 1–6 months of age, the prevalence of vitamin B12 deficiency was 63.7%. The deficiency was ubiquitous and on multivariate analysis, there were no identifiable clinic sociodemographic risk factors. Majority of the infants was normally nourished (91.3%) and their mothers were non-vegetarian (86.6%). This suggests that even among the non-vegetarians in our country the quality/quantity may not be sufficient to meet the recommended daily allowance. Though anaemia was present in 57% of the infants, it was predominantly microcytic hypochromic anaemia (70%) and there was no significant relationship between anaemia and serum B12 deficiency suggesting that the anaemia could be due to other coexisting nutritional deficiencies. The high prevalence of microcytic hypochromic anaemia, probably due to iron deficiency among

exclusively breast fed infants has been previously reported [11].

A previous study from India among EBF infants has shown a similar prevalence (57%) of B12 deficiency [7] while, in other countries it is far less (17–22%) [12]. Studies from India in older children show a lower prevalence (14–22%) suggesting that, with the introduction of complementary feeds, the vitamin B12 status improves [13–15]. Greibe *et al.* have shown that the concentration of vitamin B12 in breast milk declines progressively and by the 4th month of infancy, it is insufficient to meet the physiological demands of the exclusively breastfed infant. Hence we stratified our subject population as ≤ 3 months of age and > 3 months but did not find any significant difference in the prevalence of B12 deficiency in the two groups [16]. We postulate that, due to the wider prevalence and greater severity of B12 deficiency among the mothers in our population, their breast milk content of B12 is low even earlier.

The clinical implications of this deficiency in asymptomatic children needs further exploration. Several authors have shown the association between B12 deficiency and neurodevelopmental delay and regression in infants [13, 17–19]. Though improvement after B12 supplementation has been reported by some authors [21, 22], it is inconsistent and incomplete [20]. The other manifestations reported include skin hyperpigmentation, growth retardation, weakness, hypotonia, decreased activity disinterest in the environment [21, 22] and infantile tremor syndrome [23, 24]. The association between B12 deficiency and haematological abnormalities reported in adults is not common in children [25] and hence even children without anaemia are at risk of B12 deficiency.

A limitation of our study was that serum methylmalonic acid, a functional marker of vitamin B12 deficiency was not measured. Serum vitamin B12 alone may not be the best marker of deficiency. We did not study the B12 levels in the mothers or in the breast milk. Also, the mothers categorized as non-vegetarian diet were unable to accurately quantify their intake making it difficult to study the relationship between maternal diet and infants serum B12 level.

TABLE 1. Overview of variables in study subjects

Variables	Mean (SD)
Infant age	3.057 (1.0275)
Serum vitamin B12 level	199.91 (112.523)
Infant haemoglobin	10.795 (1.2581)
Maternal age	24.05 (3.642)
Maternal Hb at delivery	11.1852 (0.90765)

TABLE 2. Risk factors for low serum vitamin B12 levels in exclusively breast fed infants

Variables	Low B12 ≤200 pg/dl N = 95 (63.7%)	Normal B12 >200 pg/dl N = 54 (36.2%)	Total 149	Unadjusted OR (CI)	p value	Adjusted OR (CI)	p value
Age ≤3 months	57 68.7%	26 31.3%	83 55.7%	1.615 (0.824–	0.162		
Age >3 months	38 57.6%	28 42.4%	66 44.3%	3.168)			
MAM	13 100%	0 0%	13 8.7%	1.659 (1.447–1.901)	0.004	0.00000067 (0.00)	0.998
Anaemia in infant	58 68.2%	27 31.8%	85 57%	1.568 (0.799–3.076)	0.190		
Mother's age at conception ≤20 years	24 66.7%	12 33.3%	36 24.1%	1.183 (0.536–2.610)	0.677		
Maternal anaemia at delivery 7–11 g/dl	40 76.9%	12 23.1%	52 34.8%	2.545 (1.191–5.442)	0.014	1.640 (0.706–3.809)	0.998
SES ≤ Class 2	30 55.6%	24 44.6%	54 36.2%	0.577 (0.290–1.150)	0.116		
Vegetarian diet	20 100%	0 0%	20 13.4%	1.720 (1.486–1.991)	0.000	0.00000833 (0.00)	0.250

This study has important implications in that apparently healthy, term infants without risk factors could be having B12 deficiency with risk of neuro-developmental impairment during a period of rapid brain growth. Hence, in resource poor countries, there is an urgent need to supplement B12 in EBF infants, either directly or through their mothers.

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