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RESEARCH ARTICLE

REVISED Association of maternal and cord vitamin B12 levels with anthropometry in term neonates born to malnourished mothers in coastal South India [version 2; peer review: 2 approved]

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Abstract

Background

Malnourished pregnant women are at increased risk of micronutrient deficiency. We assessed the vitamin B12 status in both malnourished and normally nourished pregnant women and their neonates. Additionally, we studied the association between maternal B12 levels, cord B12 levels and neonatal anthropometry.

Methods

This cross-sectional study enrolled 63 malnourished and 63 normally nourished mothers and neonates. Maternal and cord blood samples were collected at the time of delivery for estimation of vitamin B12 levels. Maternal and cord vitamin B12 levels were compared using the Mann–Whitney U test. Neonatal anthropometry was correlated with maternal and cord B12 levels using Spearman's correlation. Data were analyzed using SPSS version 25.

Results

Mean maternal age was 26.58 yrs. The median cord B12 levels were lower than the maternal B12 levels. Maternal B12 levels showed a strong positive correlation with cord B12 levels (rho = 0.879; p <

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0.001). Maternal ($p < 0.001$) and cord ($p < 0.001$) vitamin B12 levels were significantly lower in the malnourished group than in the normally nourished group. In malnourished group, 66.8% mothers and 95.2% neonates were Vitamin B12 deficient, whereas 1.5% mothers and 4.7% neonates were vitamin B12 deficient in normally nourished group. In the malnourished group, maternal B12 levels were positively correlated with birth weight (rho 0.363, $p = 0.003$) and length (rho 0.330, $p =0.008$), whereas cord B12 levels were positively correlated with birth weight in the normally nourished group. (rho 0.277 $p= 0.028$)

Conclusion

High rates of vitamin B12 deficiency were observed in malnourished mothers and neonates. There was a positive correlation between birth weight, length, and maternal vitamin B12 levels in malnourished mothers. These findings emphasize the need to address maternal malnutrition and vitamin B12 deficiency to improve neonatal health.

Keywords
Health, low birth weight, malnourished, neonate, pregnant women, umbilical cord , vitamin B12

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REVISED **Amendments from Version 1**

Introduction was restructured to provide a clear overview of topic and the research objectives.

Discussion was restructured to provide more comprehensive analysis of the findings.

New data were added to tables to provide more detailed information and support research findings.

Tables were rearranged for better readability.

Any further responses from the reviewers can be found at the end of the article

Introduction

Vitamin B12, also known as cobalamin, is a micronutrient essential for cell growth and differentiation.[1] This essential vitamin plays a crucial role in various biochemical processes, particularly in the synthesis of DNA, in conjunction with folic acid.[2] Adequate levels of vitamin B12 during early childhood promote growth and prevent cognitive impairment. Vitamin B12 is commonly found in animal-based food products such as dairy, fish, eggs, and poultry. Consuming these foods is an effective way to acquire vitamin B12 from the diet.[3]

While the vegetarian population is at a heightened risk of deficiency, it is also prevalent among non-vegetarians.[3] Vitamin B12 deficiency is a major health concern worldwide. Pregnant women and young children are at an increased risk of facing this deficiency.[4] Regional disparities in the occurrence of vitamin B12 deficiency throughout pregnancy have been observed, indicating varying levels of risks across different regions.[5]

Inadequacy of vitamin B12 in pregnancy has been linked to unfavourable pregnancy outcomes, including spontaneous abortions, stillbirth, neural tube defects, intrauterine growth restriction, low birth weight, and premature delivery.[6–10] Deficiency of vitamin B12 is also implicated in increased risk of gestational diabetes mellitus.[11] Vitamin B12 levels in expectant mothers are thought to influence the vitamin B12 status of the fetus and infant. Vitamin B12 deficient mothers may produce breast milk with insufficient levels of vitamin B12, and their exclusively breast-fed infants may develop symptoms in the postnatal period. These infants may have apathy, anorexia, irritability, failure to thrive, delayed or regression of milestones, pancytopenia, hypotonia, and seizures. Early symptoms may be nonspecific and pose challenges for identification.[12–16] A systematic review by Rogne et al. reported no clear linear relationship between birth weight and maternal vitamin B12 levels during pregnancy. However, deficiency was linked to a higher number of neonates born with low birth weight.[17] The association between maternal, neonatal, and infant vitamin B12 levels has been heterogenous.[12–16]

Pregnant women experiencing malnutrition face a heightened risk of micronutrient deficiencies, pregnancy complications, and perinatal outcomes.[18] There are limited data regarding the occurrence of vitamin B12 deficiency in malnourished expectant mothers and its impact on neonatal outcomes. We aimed to assess the vitamin B12 status in malnourished and normally nourished pregnant women and to study the association between maternal B12 levels and cord B12 levels and neonatal anthropometry.

Methods

Study setting and period

This cross-sectional study was conducted in a community maternity facility affiliated with the Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Manipal, India. The study was conducted between August 2020 and July 2021.

Study design

This cross-sectional study followed the standard Strengthening Reporting of Observational Studies in Epidemiology (STROBE) statement guidelines.[19] The reporting guidelines contain the completed STROBE checklist.[20]

Study participants

The first group consisted of malnourished mothers and their neonates. The other group was comprised of normally nourished mothers and their neonates.

Inclusion criteria

All term neonates born to normal and malnourished mothers under singleton pregnancies.

Exclusion criteria

(a) Pregnant women <18 years and > 35 years.

(b) Pregnancy-induced hypertension/preeclampsia.

(c) Maternal history of smoking and substance abuse.

(d) Women with chorionic disease.

(e) Women with diabetes mellitus.

(f) Women with pre-existing cardiovascular hypertension or renal disorders.

(g) Pregnant women receiving multivitamin supplements other than routine iron and folic acid.

(h) Severe anemia.

(i) Blood transfusions within 3 months.

(j) Neonates with major congenital anomalies.

Sample size and technique

The sample size was determined based on a prior study by Finkelstein JL, et al., which reported the extent of vitamin B12 deficiency in South Indian pregnant women was 51%.[21] With 95% confidence interval, 80% power, and a 5% level of significance. The sample size was calculated to be 63 for each group. A random sampling technique was used to reduce selection bias.

Operational definition

Malnourished mother: A mother with a pre-pregnancy weight of less than 45 kg documented in antenatal records.[22]

Small for gestational age (SGA) is defined as birth weight < 10^{th} percentile for gestation.[23]

Vitamin B12 level[24]

Normal	>300 pg/ml
Insufficiency	200-300 pg/ml
Deficiency	<200 pg/ml

Ethics and data collection

Our research adhered to the ethical principles of world medical association Helsinki declaration of 1964 and later amendments. The study was carried out after obtaining permission from the Institutional Ethical Committee of KMC Mangalore, Manipal Academy of Higher Education (approval number IEC KMC MLR-08/2020/249 letter dated 20/08/2020), and necessary permissions were sought from hospital authorities. Based on the inclusion and exclusion criteria, suitable subjects were approached and provided with a participant information sheet. The purpose of the study was explained in their native language, and consent was obtained from those willing to participate. After obtaining consent, the participants were recruited for the study. Participation in the study was voluntary, and the complete anonymity of the research participants was maintained. We ensured confidentiality of the collected data.

Maternal and neonatal details were obtained from antenatal and hospital records. Neonatal anthropometric measurements were performed by an investigator. Weight was measured to the nearest 0.01 kg using an electronic weighing machine, while the length of the newborn was estimated using an infantometer to the nearest to 0.1 cm, and the circumference of the head was calculated using a non-stretchable tape nearest to 0.1 cm. Based on birth weight and gestational age, the babies were categorized as Large, Small, and Appropriate for Gestational Age. Maternal and neonatal details were documented using a semi-structured pretested pro forma.

Sample collection
At the time of delivery, approximately 4 ml of maternal venous blood was collected in plain glass tubes, and 4 ml of blood from the umbilical cord was directly obtained via venipuncture of the umbilical vein. The specimens were then immediately placed on ice. Centrifugation was performed at a rate of 4000 revolutions per minute for 5 min. Subsequently, the plasma was separated and stored at -80°C pending analysis.

Test procedure
Serum vitamin B12 levels were measured using a vitamin B12 ELISA kit (Epitope Diagnostics, San Diego, CA 92121, USA) at the Department of Biochemistry, KMC Centre for Basic Sciences, Bejai, Mangalore. In this assay, antibodies specific to vitamin B12 were bound to the surface of a microtiter plate. Standards or samples containing vitamin B12 and conjugated vitamin B12-peroxidase were added to the wells of a microtiter plate. Free vitamin B12 and enzyme labelled vitamin B12 compete for the binding sites on the antibodies. Following one hour of incubation at room temperature, the wells were washed with a diluted washing solution to eliminate any unbound materials. Subsequently, the substrate solution was added and incubated for 20 min, leading to the development of a blue color. This color development was inhibited by adding a stop solution, after which it turned yellow. Color intensity was measured photometrically at 450 nm. The concentration of vitamin B12 displayed an inverse relationship with the color intensity observed in the test sample.

Outcome variables
Percentage of maternal and cord B12 levels in normal, insufficient and deficient category.

Relation between maternal and cord B12 level and neonatal anthropometry.

Statistical analysis
The collected data were entered into the Statistical Package for IBM (SPSS) Statistics for Windows version 25.0. Armonk, NY: IBM Corp. for analysis. Anthropometric and demographic data were expressed as means and standard deviations or proportions. Vitamin B12 levels are expressed as medians and interquartile ranges. Maternal and neonatal characteristics were compared in both groups using the independent sample t-test and chi-square test. Vitamin B12 levels were compared between groups using the Mann–Whitney U test. Correlations between maternal and cord B12 levels and neonatal anthropometries were assessed using the Spearman correlation test.

Results
Of the 126 pregnant women enrolled, 63 were malnourished and 63 were normal nourished. The mean age of the study subject was 26.58 (± 3.79) years. The mean pre-pregnancy weight was 48.68 (± 6.17) kg. Among these women, 75 (59.5%) were primigravidas. mixed diet was followed by 117 (92.9%) women, and all women consumed milk. Among the neonates, 64 (50.8%) were male and 62 (49.2) were female. The mean gestational age of the neonates was 38.39 (± 1.19) weeks. Mean birth weight was 2.77 (± 0.35) kg and 22 (17.5%) neonates belonged to small for gestation age (SGA) category. Maternal and neonatal characteristics are shown in Table 1.

Table 1. Maternal and neonatal characteristics.

Parameter	mean (SD) or %
Maternal characteristics	
Age, yrs	26.58 (3.79)
Height, cm	157.83 (4.20)
Pre-pregnancy weight, kg	48.68 (6.17)
Maternal weight at delivery, kg	56.96 (6.34)
BMI kg/m^2	19.35 (2.79)
Weight gain during pregnancy, kg	8.30 (0.85)
Gravida* (%)	
Primigravida	75 (59.5)
Multigravida	51 (40.5)

Table 1. *Continued*

Parameter	mean (SD) or %
Diet* (%)	
Vegetarian	9 (7.1)
Mixed diet	117 (92.9)
Milk consumption* (%)	
Yes	126 (100)
No	0 (0)
Neonatal characteristics	
Gestational age (weeks)	38.39 (1.19)
Birth weight (kg)	2.77 (0.35)
Gestational weight category* (%)	
Appropriate for Gestational Age	104 (82.5)
Small for Gestational Age	22 (17.5)
Length (cm)	48.87 (0.99)
Head circumference (cm)	33.94 (0.97)
Gender* (%)	
Female	62 (49.2)
Male	64 (50.8)

SD standard deviation.
*Values expressed as %.

Comparison of maternal anthropometric measurements among malnourished and normal nourished mothers is depicted in Table 2. Birth weight, length, and head circumference of neonates born to malnourished mothers were lower than those of neonates born to mothers with normal nourishment. Sixteen (25.4%) neonates of malnourished mother group were SGA. A comparison of the anthropometric parameters of neonates born to malnourished and normal-nourished mothers is shown in Table 2.

Table 2. Maternal and neonatal characteristics among normally nourished and malnourished mothers.

Parameter	Normal nourished mothers Mean (SD)	Malnourished mothers Mean (SD)	P-value
Maternal characteristics			
Height	157.17 (4.87)	158.48(3.34)	0.083
Pre-pregnancy weight	53.839 (4.72)	43.53(0.90)	<0.001
Weight at delivery	62.22 (4.79)	51.7 (1.33)	<0.001
BMI at delivery	21.35 (2.66)	17.37 (0.82)	<0.001
Weight gain during pregnancy	8.42 (0.89)	8.18 (0.74)	0.106
Neonatal characteristics			
Gender* (%)			
Female	29 (46)	33 (52.4)	0.476
Male	34 (54)	30 (47.6)	
Gestational age, weeks	38.32 (1.11)	38.46 (1.28)	0.504
Birth weight, kg	2.95 (0.38)	2.57 (0.19)	<0.001

Table 2. *Continued*

Parameter	Normal nourished mothers Mean (SD)	Malnourished mothers Mean (SD)	P-value
Birth weight category* (%)			
AGA	57 (90.5)	47 (74.6)	0.033
SGA	6 (9.5)	16 (25.4)	
Length, cm	49.48 (0.72)	48.25 (0.84)	<0.001
Head circumference, cm	34.19 (1.12)	33.68 (0.71)	<0.001

SD standard deviation.
*Values expressed as %.

Table 3. Maternal and cord vitamin B12 levels.

Vitamin B12 levels (pg/ml)	Median (Interquartile range)
Cord	191.5 (89.63-358.75)
Maternal	257.9 (167-505)

The median cord B12 value was considerably lower than the maternal B12 value, as shown in Table 3. There was a strong positive correlation between maternal and cord B12 levels (rho 0.829, p <0.001). Spearman correlation test for maternal BMI and vitamin B12 levels showed a positive correlation between the two measures (rho 0.590, p <0.001). Both maternal and cord B12 levels were significantly lower in the malnourished group than in the normal-nourished group (Table 4). In malnourished group, 66.8% mothers and 95.2% neonates were vitamin B12 deficient. In normal nourished group, only 1.5% mothers and 4.7% neonates were deficient. The maternal and cord B12 levels in both groups are shown in Table 4. Odd's ratio for malnourished mothers to have vitamin B12 deficiency was 160 (95% CI 39.43, 649.27). In the malnourished group, the maternal B12 level showed a positive correlation with birth weight (rho 0.363, p = 0.003) and length (rho 0.330, p = 0.008), whereas in the normally nourished group, the cord B12 level was positively correlated with birth weight (rho 0.277 p = 0.028) (Table 5), respectively.

Table 4. Maternal and cord vitamin B12 level and categories in normally nourished and malnourished mothers.

Parameter	Normal nourished mothers Median (IQR) or n (%)	Malnourished mothers Median (IQR) or n (%)	P-value
Vitamin B12 Levels (pg/ml)			
Maternal	499 (357-748)	168 (123-208)	<0.001
Cord	352 (259.60-438)	93 (57.9-119)	<0.001
B12 Categories* n (%)			
Maternal Vitamin B12			
Normal	56 (89)	3 (4.7)	<0.001
Insufficient	6 (9.5)	18 (28.5)	
Deficient	1 (1.5)	42 (66.8)	
Cord Vitamin B12			
Normal	43 (68.3)	2 (3.2)	**<0.001**
Insufficient	17 (27)	1 (1.6)	
Deficient	3 (4.7)	60 (95.2)	

IQR Inter quartile range.
*Values expressed as %.

Table 5. Correlation of maternal,cord vitamin B12 and neonatal anthropometry in normal nourished and malnourished women.

Parameter	Normally nourished				Malnourished			
	Maternal B12		Cord B12		Maternal B12		Cord B12	
	rho	P value	rho	P value	rho	P value	rho	P value
Birth weight	0.228	0.073	0.277	0.028	0.363	0.003	0.133	0.298
Length	0.064	0.621	0.007	0.956	0.330	0.008	0.011	0.934
Head circumference	0.049	0.703	0.021	0.869	0.09	0.483	0.166	0.193

Discussion

Vitamin B12 deficiency is widespread globally, and affects various populations. A study by Ramírez-Vélez et al in a sample representative of pregnant Colombian women, revealed that 18.6% of the population was deficient in vitamin B12 and 41.3% exhibited insufficient vitamin B12.[25] In a nutritional intervention study, that enrolled moderately malnourished Malawian mothers, 20.9% of the participants were deficient in vitamin B12.[26]A study by Yildizdas et al in Turkey reported high rate of maternal vitamin deficiency of 96.3%.[27]

There is a significant occurrence of vitamin B12 deficiency in Indian women during pregnancy. Studies from western and northern parts of India have reported the highest occurrence of vitamin B12 deficiency during pregnancy, ranging from 70–74%.[5] A community based cross sectional study by Barney et al., enrolling pregnant women in rural South India, reported an extent of 55% vitamin B12 deficiency. They found obesity and being in the first trimester of pregnancy to be independent risk factors.[28] A study conducted by Katre et al. in Pune, which recruited pregnant women at 17 weeks of gestation, indicated that approximately 80% of rural and 65% of urban women exhibited low Vitamin B12 status.[29] A prospective cohort study involving pregnant women aged 18-45 years from urban hospitals in Bangalore reported low plasma vitamin B12 levels in 48.6% of women.[30] In the present study, 66.8% of women and 95.2% of neonates in the malnourished group exhibited vitamin B12 deficiency.

Despite general assumption that non-vegetarian diet provides sufficient vitamin B12, recent research has reported vitamin B12 deficiency in non-vegetarian population.

A review by Sukumaran et al. suggested that even within non-vegetarian populations, vitamin B12 insufficiency during pregnancy is frequently observed.[31] Similar results were observed in our study, where there was a high prevalence of maternal vitamin B12 deficiency in a coastal community with high fish consumption. Food taboos targeting pregnant and postpartum women are widely prevalent in India and Southeast Asian countries which may contribute to this deficiency.[32,33]

Vitamin B12 levels change during pregnancy across the trimesters. Concentrations of vitamin B12 decreased from the first trimester to the third trimester, as reported by Sukumaran et al.[31] Similar observations were reported by Sobowale et al. from Bangladesh.[34] A cohort study involving healthy pregnant adolescent women from New York revealed decreasing levels of maternal serum cobalamin levels from mid gestation to delivery.[35] Hence cord B12 levels may not reflect maternal levels across the trimesters.

Low maternal vitamin B12 levels have resulted in lower neonatal B12 status as reported by previous studies. A study by Adaikalakoteswari et al. found that the offspring of mothers with low B12 levels exhibited significantly lower levels of vitamin B12 than did those of mothers with normal B12 levels.[12] A Study by Finkelstein e t al., who recruited pregnant women at approximately 12 weeks gestation from South India, revealed that vitamin B12 deficiency was prevalent in approximately 63% of women during early pregnancy.[35] This deficiency serves as predictor of neonatal vitamin B12 status. Specifically, lower maternal serum vitamin B12 levels were associated with a two-fold increased risk of neonatal vitamin B12 deficiency at birth.[36] The present study also reported a positive correlation between maternal and cord vitamin B12 levels, with cord B12 levels being lower than maternal B12 levels. In contrast, a study by Yildizdas et al. involving Turkish mothers and neonates, reported that micronutrient levels in the cord blood surpassed maternal levels.[27]

The relationships between maternal vitamin B12 and, cord B12 and neonatal anthropometry are heterogenous. In a study by Yildizdas et al., there was a negative correlation between birth weight, head circumference, and cord blood Cbl levels. Interestingly, maternal Cbl levels did not show any correlation with neonatal anthropometry.[27] In a retrospective study by Yuan et al. in Eastern China involving 11,549 pregnant women, it was found that elevated maternal serum vitamin B12 levels were correlated with higher birth weight and an increased risk of new born being large.[7] Sukumar et al. reported that there is no consistent relationship between vitamin B12 sufficiency and LBW.[31] MAASTHI birth cohort study by Deepa et al. in South India revealed no association between vitamin B12 status and birth weight.[30]

Hay et al. reported a negative association between cord Cbl and birth weight. The heaviest babies had lower cord Cbl levels. A similar negative association was also observed between birth length and head circumference.[37] A secondary analysis of 709 Canadian mother newborn dyads by Tan et al. revealed that maternal serum vitamin B12 biomarkers did not show any association with birth weight or head circumference.[38] In the present study, maternal B12 levels in malnourished women showed a positive correlation with birth weight (rho 0.363, p = 0.003) and length (rho 0.330, p = 0.008) but not with head circumference.

Limitations: This study was conducted in a tertiary care hospital, which may restrict the generalizability of its findings to a broader community. Maternal vitamin B12 levels measured only at the time of delivery may not reflect fluctuations throughout pregnancy. A smaller sample size raises concerns regarding the applicability of the results. The presence of coexisting micronutrient deficiencies in malnourished women poses a risk of confounding bias in the interpretation of results.

In conclusion, the present study revealed high rates of vitamin B12 deficiency in malnourished mothers and their neonates. Additionally, there was a positive correlation between birth weight, length, and maternal vitamin B12 levels in malnourished mothers. These findings emphasize the need to address maternal malnutrition and vitamin B12 deficiency to improve neonatal health.

Ethical considerations and consent

Institutional ethical approval was obtained from the ethics committee of Kasturba Medical College Mangalore (IEC KMC MLR-08/2020/249). Permission from the medical superintendent of the hospital was obtained prior to the study. A patient information sheet was provided and the purpose of the study was explained to the parents. Written informed consent was obtained from parents or legal guardians following the Helsinki Declaration. Participation in the study was voluntary, and the complete anonymity of the research participants was maintained. We ensured confidentiality of the collected data.

Data availability

Underlying data

Association of maternal and cord vitamin B12 levels with anthropometry in term neonates born to malnourished mothers in Coastal South India. figshare. Dataset. https://doi.org/10.6084/m9.figshare.25622415.[20]

Reporting guidelines

Figshare: STROBE checklist for ‘Association of maternal and cord vitamin B12 levels with anthropometry in term neonates born to malnourished mothers in Coastal South India.’ https://doi.org/10.6084/m9.figshare.25622415.[20]

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Open Peer Review

Current Peer Review Status: ✔ ✔

Version 2

Reviewer Report 02 August 2024

https://doi.org/10.5256/f1000research.169306.r306042

✔

Noor Rohmah Mayasari
Nutrition, Universitas Negeri Surabaya, Surabaya, East Java, Indonesia

No further comments

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Nutritional epidemiology, anemia, food security

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 01 August 2024

https://doi.org/10.5256/f1000research.169306.r306041

✔

Rathika Damodara Shenoy
American University of Antigua, Antigua, USA

The author in the revised version has addressed my queries. No further comments.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Perinatology, Metabolic disorders, Dysmorphology

I confirm that I have read this submission and believe that I have an appropriate level of

expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 29 June 2024

https://doi.org/10.5256/f1000research.165288.r289090

?

Noor Rohmah Mayasari
[1] Nutrition, Universitas Negeri Surabaya, Surabaya, East Java, Indonesia
[2] Nutrition, Universitas Negeri Surabaya, Surabaya, East Java, Indonesia

General comments:
The study aimed to assess vitamin B12 status in malnourished and normally nourished pregnant women and study the association between maternal B12 level and cord B12 labels and neonatal anthropometry. However, the paper hasn't discussed the findings related to the aim.

Introduction:
1. Please modify the introduction structure (the paragraph doesn't have the main sentence of each). Some paragraphs are too short (less than three sentences)

Methods:
1. Please clarify the study design, is there a case-control or cross-sectional study?
2. Why exclude severe anaemia, is there any specific reason?
3. Malnourished is define as a pre-pregnancy body weight less than 45kg. Why use body weight rather than pre-pregnancy body mass index?

Results:
1. Explain the reason why in the table presentation authors use different display outcomes (table 2 (mean and SD), table 3 (median and interquartile range).
2. Table 2 expresses as (%) or (mean and SD)?
3. Tables 2-5can be merged as 1 table and Tables 6-7 can be merged as 1 table.
4. From the study authors can analyze RR or OR

Discussion:
1. What Is the key finding of this study?
2. Please modify the discussion structure (the paragraph doesn't have the main sentence of each). Some paragraphs are too short (less than three sentences).
3. The data shows that maternal vitamin B12 positively correlated with Birth weight and length among malnourished women. However, this correlation is not significant with cord vitamin B12, what is that mean and implication?
4. Are there any maternal characteristics that effect the main outcome as confounding factors?

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Nutritional epidemiology, anemia, food security

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 17 Jul 2024

SUCHETHA S RAO

Reviewer Comment:
General comments:
The study aimed to assess vitamin B12 status in malnourished and normally nourished pregnant women and study the association between maternal B12 level and cord B12 labels and neonatal anthropometry. However, the paper hasn't discussed the findings related to the aim.

Author reply : Thank you for reviewing our article and giving valuable suggestions. We have modified the article as per your suggestions. We have added additional data related to aim.

Reviewer Comment:
Introduction:
1.Please modify the introduction structure (the paragraph doesn't have the main sentence of
each). Some paragraphs are too short (less than three sentences)

Author reply : Introduction has been modified as per the suggestion
First paragraph- Role of Vitamin B12 in health , sources
Second paragraph- Deficiency and health burden , prevalence and regional disparities of deficiency
Third paragraph -Vitamin B12 deficiency adverse outcomes in pregnancy , and effect of maternal deficiency on child health
Fourth paragraph - Need for the present study and aims of the study
----

Reviewer Comment:
Methods

1. Please clarify the study design, is there a case-control or cross-sectional study?
Author reply Cross sectional study
2. Why exclude severe anaemia, is there any specific reason?
Author reply Severe anemia itself can lead to low birth weight babies.
3 .Malnourished is define as a pre-pregnancy body weight less than 45kg. Why use body weight rather
than pre-pregnancy body mass index?

Author reply : Antenatal records had pre pregnancy weight documented not BMI ,hence we chose weight criteria for defining malnourished mother
----

Reviewer Comment:
Results:
1.Explain the reason why in the table presentation authors use different display outcomes (table 2 (mean and SD), table 3 (median and interquartile range).

Author reply :
Table 2 mentions about anthropometric measures which followed normal distribution , hence we used mean and SD
Table 3 mentions about vitamin B12 levels which did not follow normal distribution , hence used Median and Interquartile range
----

Reviewer Comment:
2.Table 2 expresses as (%) or (mean and SD)?

Author reply :
Expressed as Mean and SD or as (%). values marked * are expressed as (%)

Reviewer Comment:
3.Tables 2-5 can be merged as 1 table and Tables 6-7 can be merged as 1 table.

Author reply :
We have merged table 4 and 5 as one table (new table no 4)
We have merged table 6 and 7 as one table (new table no 5)

Reviewer Comment:
4.From the study authors can analyze RR or OR

Author reply :
We have calculated OR. OR for malnourished mothers to have vitamin B12 deficiency is 160 (95% CI 39.43, 649.27)

Reviewer Comment:
Discussion:
1.What Is the key finding of this study?

Author reply :
1. A significant proportion, about 53%, of women in the study population have insufficient or deficient B12 status despite a mixed diet
2. The median cord B12 value was considerably lower than the maternal B12 value and there was a strong positive correlation between maternal and cord B12 values
3. Both maternal and cord B12 levels were significantly lower in the malnourished group than in the normal-nourished group
4. In malnourished group , maternal vitamin B12 positively correlated with Birth weight and length. Cord B12 showed no correlation with neonatal anthropometry
----
Reviewer Comment:
2. Please modify the discussion structure (the paragraph doesn't have the main sentence of each). Some paragraphs are too short (less than three sentences).

Author reply :
We have modified the discussion as per the suggestion

Reviewer Comment:
3. The data shows that maternal vitamin B12 positively correlated with Birth weight and length among malnourished women. However, this correlation is not significant with cord vitamin B12, what is that mean and implication?

Author reply :
Maternal Vitamin B12 levels during pregnancy may directly influence growth of fetus , leading to higher birth weight and increased length. This implies that adequate maternal Vitamin B12 level is crucial for fetal growth. Low levels of maternal vitamin B12, at certain stages of gestation, can lead to adverse outcomes, such as low birth weight and shorter neonates. Previous studies have reported declining concentrations of maternal vitamin B12 from first trimester to the third trimester. Cord blood Vitamin B12 levels reflect status of

Vitamin B12 of neonate at birth. Absence of correlation with cord Vitamin B12 means that Cord Vitamin B12 may not be a direct measure of ongoing maternal B12 influence during pregnancy. Critical periods of Vitamin B12 influence may occur at certain stages of gestation which may not be captured by Cord blood B12 value. This implies the need to maintain adequate maternal Vitamin B12 levels throughout pregnancy for better neonatal outcome.

Reviewer Comment:
4. Are there any maternal characteristics that effect the main outcome as confounding factors?

Author reply : Co existing micronutrient deficiencies in malnourished mothers may effect the main outcome as confounding factors. This has been mentioned in limitations.

Competing Interests: None

Reviewer Report 22 June 2024

https://doi.org/10.5256/f1000research.165288.r289097

Rathika Damodara Shenoy
[1] American University of Antigua, Antigua, USA
[2] American University of Antigua, Antigua, USA

The cross-sectional study compares the maternal and cord blood B12 levels in normally nourished and malnourished mothers at term. The authors report lower maternal and cord blood B12 values in the malnourished group and a strong positive correlation between the measures. The correlations between B12 values and newborn anthropometry are weak to none.
The results reveal that a significant proportion, about 53%, of women in the study population have insufficient or deficient B12 status despite a mixed diet. It may be worthwhile to compare the maternal demography of the two groups (like Table 2 on newborn data). The authors can investigate if maternal BMI and B12 correlation will provide additional information. The limitations are listed by the authors.
The main takeaway from the study is the high prevalence of maternal B12 deficiency in a coastal fish-eating community. Food taboos targeting pregnant and postpartum women are widely prevalent in India and South Asian countries.[1,2] The authors should investigate qualitative and quantitative diet information, the coexistence of iron deficiency, and the clinical relevance in future studies with a larger sample size.

References

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2. Kareem O, Mufti S, Nisar S, Tanvir M, et al.: Prevalence of Thiamine Deficiency in Pregnancy and its impact on fetal outcome in an area endemic for thiamine deficiency.*PLoS Negl Trop Dis*. 2023; **17** (5): e0011324 PubMed Abstract | Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Perinatology, Metabolic disorders, Dysmorphology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 17 Jul 2024

SUCHETHA S RAO

Review comment : The cross-sectional study compares the maternal and cord blood B12 levels in normally nourished and malnourished mothers at term. The authors report lower maternal and cord blood B12 values in the malnourished group and a strong positive correlation between the measures. The correlations between B12 values and newborn anthropometry are weak to none.
The results reveal that a significant proportion, about 53%, of women in the study population have insufficient or deficient B12 status despite a mixed diet. It may be worthwhile to compare the maternal demography of the two groups (like Table 2 on newborn data).
Author reply : Thank you for reviewing our article and giving valuable suggestions. We have modified the article as per your suggestions. We have compared the maternal

demography and details are added to table 2 along with newborn data

Review comment : The authors can investigate if maternal BMI and B12 correlation will provide additional information.
Author reply : Spearman correlation test for BMI and Vitamin B12 level was done. There was a positive correlation between the two measures. (rho 0.590 , p <0.001) This observation has been added to result.

Review comment :The limitations are listed by the authors.
The main takeaway from the study is the high prevalence of maternal B12 deficiency in a coastal fish-eating community. Food taboos targeting pregnant and postpartum women are widely prevalent in India and South Asian countries.1,2
Author reply : Thank you for highlighting the significance of food taboos for possible contribution towards maternal Vitamin B12 deficiency along with references. We have added this to discussion.

Review comment The authors should investigate qualitative and quantitative diet information, the coexistence of iron deficiency, and the clinical relevance in future studies with a larger sample size.
Author reply We will plan for future studies

Competing Interests: None