Experimental
&
Clinical Article

Reassessing Nutritional Supplementation Program: The Impact of Maternal Vitamin B12 Levels on Feto-Maternal Outcomes

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ABSTRACT

OBJECTIVES: In India, despite the high prevalence of vitamin B12 (vit B12) deficiency, the current public health policy focuses solely on iron and folic acid supplementation as a measure to prevent anaemia. Vit B12 is an important cofactor for enzymatic reactions related to DNA synthesis, folate, and cell metabolism. While folate deficiency has been acknowledged in not only the prevention of neural tube defects and other adverse pregnancy outcomes, the effects of low vit B12 status have not been well defined. It is becoming recognized that there is a much higher prevalence of B12 deficiency than earlier suspected, and more of its effect on maternal and fetal health is being reported. The current study was planned as an observational study to correlate the effects of vit B12 deficiency with respect to various feto-maternal outcomes.

STUDY DESIGN: In this prospective cohort study a total of 300 antenatal women were screened for vit B12 levels in the late first trimester and were followed up to find out co-existent folic acid deficiency, development of pregnancy complications like anemia, gestational hypertension/pre-eclampsia, fetal growth restriction (FGR), second-trimester abortions, gestational diabetes mellitus (GDM) and preterm deliveries.

RESULTS: It was found that most of the women with low B12 levels were vegetarian and had co-existent folic acid deficiency. They had a statistically significant increase in the incidence of anemia, pre-eclampsia, gestational diabetes, (small for gestational age) SGA/FGR, and preterm births.

CONCLUSION: The study suggests that low levels of vit B12 increase the likelihood of adverse feto-maternal outcomes. Further research involving larger sample sizes is necessary to confirm these findings and determine if vit B12 supplementation should be included in pregnancy nutritional supplements, especially in vegetarian women.

Keywords: B12 deficiency, Maternal nutrition, Vit B12, Feto-maternal outcome, Vit B12 supplement

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Introduction

Maternal undernutrition predisposes to a range of adverse pregnancy outcomes including maternal anemia, early pregnancy loss, hypertensive disorders of pregnancy, fetal congenital anomalies, poor fetal growth, and perinatal morbidity and mortality. Poor fetal growth increases the risk of long-term ill-health in the offspring including increased risk of non-communicable diseases. Therefore, improving maternal nutrition during pregnancy is an important focus of routine antenatal care and public health initiatives.

Traditionally vit B12 deficiency is equated to pernicious anaemia (a genetic-immunological condition of intrinsic factor deficiency leading to poor absorption) and neurological problems. There is an increasing recognition of the importance of maternal micronutrients (iron, vit B12, folate, vitamin

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C, vitamin D, pyridoxine, and choline) in fetal growth and development. VitB12 and folate act as methyl donors in one-carbon metabolism which affects cell growth and differentiation by influencing DNA synthesis and epigenetic regulation. Therefore, they are important regulators of fetal growth. In addition, they are also important co-factors necessary to maintain normal levels of homocysteine, high levels of which are known to cause several pregnancy complications due to their pro-inflammatory properties (1,2).

However, while folic acid and iron deficiencies are being addressed, vit B12 supplementation is not a part of routine supplementation in pregnancy. The effects of low vit B12 status in reproduction are not well defined, even though there are close metabolic links between folate and vit B12. This may be partially due to evidence that severe vit B12 deficiency, as manifested in pernicious anemia, can cause infertility, but also that vit B12 deficiency is generally considered to be a deficiency of the elderly, and therefore the consequences of inadequate or marginal vit B12 status on reproduction have seldom been addressed. However, these views are being modified and it is becoming recognized that there is a much higher global prevalence of low vit B12 status among women and children than hitherto suspected. Consequently, reports of the effects of nutritional vit B12 deficiency on maternal and neonatal health are appearing more often in the literature (3).

The ultimate source of vit B12 in nature is microbes. Vit B12 enters the food chain when animals obtain vit B12 by eating microbes. Humans obtain vit B12 from animal-sourced foods (milk, eggs, fish, and meat) and bacterial contamination of water and food. Multiple studies in India have established a high prevalence of vit B12 deficiency in the population, with geographical variations that have been ascribed to both the socio-cultural practice of vegetarianism and lower socio-economic status (4). Still, the current public health policy focuses solely on iron and folic acid supplementation as a measure to prevent anemia.

Behere et al conducted a systematic review of Indian studies focusing on the relationship between vit B12 deficiency and feto-maternal outcomes (1). Using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) system, they evaluated the quality of evidence in these studies. Their review found that while observational studies supported the connection between B12 deficiency and pregnancy complications, most of the studies on recurrent pregnancy loss, pre-eclampsia, and preterm deliveries had low-quality GRADE evidence. However, the evidence for the development of gestational diabetes mellitus was of moderate quality. Notably, there was high-quality evidence to support the role of low maternal vit B12 in an increased risk of neural tube defects and low birth weight (1). They concluded that incorporating vit B12 into current nutritional programs in India can provide additional benefits for pregnancy outcomes besides managing anaemia.

Our study was designed as a prospective observational study to correlate the levels of vit B12 levels during the first trimester of pregnancy, and various feto-maternal outcomes, especially anaemia, gestational hypertension/pre-eclampsia, GDM, and FGR. A total of 302 patients were followed up from the time of booking to delivery, and the results were collated.

Material and Method

This observational prospective cohort study was conducted at the Dept of Obstetrics & Gynaecology of a tertiary care hospital. The population comprised late first-trimester patients reporting to the antenatal clinic in the department. The study included all pregnant women in their first trimester with spontaneous conception who consented to participation in the study. Patients with multiple gestations pregestational diabetes, chronic hypertension, and those who conceived through in vitro fertilization (IVF) were excluded from the study.

The institutional ethical committee of our institution approved the study protocol on 02 Sep 2020 (Approval No IEC/-8/2020/03). Informed consent of the study participants was taken and the study was conducted in accordance with the Declaration of Helsinki.

A total of 348 participants fulfilling inclusion criteria were enrolled for the study and consent was obtained. A detailed history was recorded by the researchers. Venous blood samples were collected at enrolment and analysed for routine antenatal investigations and plasma vit B12 levels. Vit B12 assay was done by chemiluminescence method using the VITROS vit B12 calibrators on the VITROS 5600 Integrated system using Intellicheck technology. The cut-off levels of vit B12, according to the machine calibration were taken at 193 pg/mL.

The candidates were categorized into two groups, one with normal plasma B12 levels and the other with low plasma B12 levels. 46 patients were lost to follow-up for various reasons and a total of 300 patients were followed up till the culmination of pregnancy, of them 9 patients suffered second-trimester abortion. Two hundred and three pregnant patients reached the period of viability. Various obstetric outcomes were recorded for all on predesigned proforma. The outcomes measured were the number of second-trimester abortions, development of anemia, gestational hypertension/pre-eclampsia, gestational diabetes mellitus, fetal growth restriction, term or preterm labor, and various neonatal outcomes like intrauterine fetal demise, fetal birth weight in centile for the period of gestation, and APGAR score at 5 min.

The collected data was analyzed using various statistical techniques to explore the features and relationships between the variables. Descriptive statistics were used to summarize and describe the data. Categorical data was analyzed using the Chi-square test to determine any associations between two variables, while the Student-t test was used for quantitative

data. A significance level of $p < 0.05$ was used to determine whether the results were statistically significant.

Results

Table I shows the distribution of vit B12 levels among 302 individuals. The results reveal that 52.31% of individuals had vit B12 levels greater than or equal to 193, while 47.68% had levels below this threshold. This suggests that there may be a significant proportion of individuals who have lower than normal vit B12 levels

Table I: *Distribution of vit B12 levels*

Vit B12 Levels	Number (n)	Percentage
≥ 193	158	52.31%
< 193	144	47.68%
Total	302	100%

The following tables present the results of a study comparing demographic characteristics, and maternal and fetal outcomes between patients with normal B12 levels and those with low B12 levels. Table I includes information on age, co-existing folic acid deficiency, food habits, gravidity, and second-trimester miscarriages, and Table II presents subgroup analysis after excluding abortions. There were no intrauterine fetal demises in other pregnancies which crossed the period of viability.

The data in Table II reveals that there were no significant differences in age or gravidity between the two groups (p=0.67 and p=0.66, respectively). A higher percentage of patients with low B12 levels had a co-existing folic acid deficiency (28.47% vs. 17.72%, p=0.02, relative risk (RR)=1.59). Additionally, vegetarianism was strongly associated with low B12 levels, with 69.93% of vegetarians having low B12 levels compared to 20.13% of non-vegetarians (p<0.001, risk ratio=3.47, 95% CI: 2.20-5.48). Since the majority of the recruitment was carried out in the late first trimester, it was not possible to document the first-trimester abortions. However, there was no significant risk of second-trimester abortion observed in the low plasma B12 group (p=0.24).

Table III presents a subgroup analysis after excluding abortions. The results indicate that patients with low B12 levels had significantly higher rates of gestational diabetes (28.98% vs. 20.64%, p=0.02), anemia (32.60% vs. 22.46%, p=0.01), pre-eclampsia (15.94% vs. 7.09%, p=0.009), and small for gestational age (SGA)/FGR (73.18% vs. 26.45%, p=0.001) compared to those with normal B12 levels.

The risk ratios (RR) calculated from the data show that patients with low B12 levels had a 1.4 times higher risk of developing gestational diabetes (RR=1.40, 95% CI 1.06-1.85), a 1.5 times higher risk of developing anemia (RR=1.53, 95% CI 1.12-2.08), a 2.3 times higher risk of developing pre-eclampsia (RR=2.30, 95% CI 1.25-4.24), and a 2.8 times higher risk of having SGA/FGR (RR=2.82, 95% CI 1.76-4.51) compared to those with normal B12 levels.

There was no significant difference between the two groups in terms of gestational hypertension (9.42% vs. 8.38%, p=0.7), term deliveries (52.17% vs. 50.96%, p=0.043), preterm deliveries at <34 weeks (5.07% vs. 3.22%), preterm deliveries 34 to <37 weeks (21.01% vs. 10.96%), and APGAR score at 5 minutes (<5: 2.17% vs. 1.2%, p=0.55).

Table II: *B12 level analysis by demographic characters, food habits, folic acid deficiency, and second-trimester abortions*

Age	Patients with normal B12 levels n=158	Patients with low B12 levels Nn=144	Total n=302	p
18-24	42 (26.58%)	43 (30.06%)	85 (28.14%)	0.67
25-29	76 (48.40%)	62 (43.05%)	138 (45.69%)	
30-35	32 (20.38%)	34 (23.77%)	66 (22%)	
>35	8 (5.09%)	5 (3.49%)	13 (4.33%)	
Gravidity				
Primigravida	72 (45.56%)	62 (43.05%)	134 (44.37%)	0.66
Multigravida	86 (54.77%)	82 (57.34%)	168 (56%)	
Co-existing Folic acid deficiency				
< 8.6 ng/ml	28 (17.72%)	41 (28.47%)	69 (22.84%)	0.02
Food habits				
Vegetarian	37 (23.41%)	100 (69.93%)	137 (45.36%)	<0.001
Non-vegetarian	33 (21.01%)	29 (20.13%)	62 (20.52%)	
2nd-trimester miscarriages	3 (1.89%)	6 (4.16%)	9 (2.98%)	0.24

Table III: *Subgroup Analysis after excluding abortions*

Variables	Patients with normal B12 levels n= 155	Patients with low B12 levels n= 138	p
Gestational diabetes	32 (20.64%)	40 (28.98%)	0.02
Gestational hypertension	13 (8.38%)	13 (9.42%)	0.7
Anaemia	31 (22.46%)	45 (32.60%)	0.01
Pre-eclampsia	11 (7.09%)	22 (15.94%)	0.009
Term deliveries	79 (50.96%)	72 (52.17%)	0.043
Preterm deliveries at <34 weeks	5 (3.22%)	7 (5.07%)	
Preterm deliveries 34 to <37 weeks	17 (10.96%)	29 (21.01%)	
SGA /FGR (weight < 10th centile)	41 (26.45%)	101 (73.18%)	0.001
(Appropriate for gestational age) AGA	102 (65.80%)	30 (20%)	
(between 10th to 90th centile)	13 (8.38%)	2 (1.44%)	
(Large for gestational age) LGA(weight > 90th centile) APGAR at 5 minutes <5	2 (1.2%)	3 (2.17%)	0.55

Discussion

It is known that Folate and vit B12 are essential for several metabolic processes in the body, involving synthesis, repair, and regulation of DNA, proteins, membranes, and neurotransmitters. Deficiency of these vitamins leads to wide-ranging multi-system abnormalities, including megaloblastic anemia, neurological disorders, and birth defects.

We observed a high prevalence of vit B12 deficiency in our study population, amounting to almost 48%. This could be attributed to the fact that most of the study population had a vegetarian diet, which predisposes to vit B12 deficiency, which has been documented by Kumavat et al (5). However, a systematic review and meta-analysis by Sukumar et al, has also shown a high prevalence of vit B12 deficiency in the non-vegetarian population as well (6). This was attributed to the lower socio-economic status of the population, who were unable to afford a non-vegetarian meal.

In a study done by Hübner et al, it was found that low levels of vit B12 had a statistically significant correlation with recurrent first-trimester abortions, the probable explanation being the increase in homocysteine levels due to B12 deficiency (7). Our study has not shown a similar significant correlation possibly because the recruitment was mostly done in the late first trimester.

Studies on the incidence of gestational hypertension/pre-eclampsia have thrown up inconsistent results. One study by Mujawar et al found lower levels of B12 in patients who developed pre-eclampsia (8). However, as per studies done by Pisal et al and Kulkarni et al, patients with pre-eclampsia had higher levels of B12 (9,10). Our study has demonstrated a higher incidence of pre-eclampsia in patients with a deficiency of vit B12, which was also statistically significant.

In addition to pre-eclampsia, we also found a higher incidence of SGA/FGR infants in patients with reduced B12 levels. This finding has been reflected in various studies carried out previously, most notably done by Wadhwani et al, Mishra et al, and Muthayya et al (11-13).

Our study revealed a significant correlation between B12 deficiency and increased incidence of GDM in comparison to patients with B12 levels >193 pg/ml. The results were consistent with the previous study by Chen et al (14). Another study by Krishnaveni et al found vit B12 deficient women had a higher incidence of gestational diabetes (15). Available evidence suggests a possible role for low maternal B12 in increasing the risk of GDM, however, reverse causality cannot be ruled out. A study by Yajnik et al, the Pune Maternal Nutritional Study did conclude that low Vit B12 concentrations in the mother predisposed to adiposity in the infant (16).

Conclusion

Our study has established a high prevalence of Vit B12 deficiency and coexisting folic acid deficiency in our population, especially in vegetarian women, and its detrimental effects on maternal and fetal health in terms of increased risk of pre-eclampsia, gestational diabetes, anemia, and FGR. The current study is not without its limitations though, primarily being a small study.

Larger studies with a more robust design would be required to understand the correlation between B12 deficiency and feto-maternal outcomes, along with variations in ethnic, geographic, and cultural groups. It would also lay down the

way forward to address this looming issue as a public health initiative.

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References

1. Behere RV, Deshmukh AS, Otiv S, Gupte MD, Yajnik CS. Maternal vitamin B12 status during pregnancy and its association with outcomes of pregnancy and health of the offspring: a systematic review and implications for policy in India. Front Endocrinol (Lausanne). 2021;12:619176. Doi: 10.3389/fendo.2021.619176. PMID: 33912132, PMCID: PMC8074968.
2. Gadgil M, Joshi K, Pandit A, Otiv S, Joshi R, Brenna JT, Patwardhan B. Imbalance of folic acid and vitamin B12 is associated with birth outcome: an Indian pregnant women study. Eur J Clin Nutr. 2014;68(6):726-9. Doi: 10.1038/ejcn.2013.289. PMID: 24448492.
3. Molloy AM, Kirke PN, Brody LC, Scott JM, Mills JL. Effects of folate and vitamin B12 deficiencies during pregnancy on fetal, infant, and child development. Food Nutr Bull. 2008;29(2 Suppl):S101-11; discussion S112-5. Doi: 10.1177/15648265080292S114. PMID: 18709885.
4. Gammon CS, von Hurst PR, Coad J, Kruger R, Stonehouse W. Vegetarianism, vitamin B12 status, and insulin resistance in a group of predominantly overweight/obese South Asian women. Nutrition. 2012; 28 (1):20-4. Doi: 10.1016/j.nut.2011.05.006. PMID:218355 92.
5. Kumawat U, Amolsing D, Thakur DR. To study the status of vitamin B12 deficiency in pregnancy and its impact on the maternal and fetal outcome. International Journal of Clinical Obstetrics and Gynaecology. 2021;5(3):243-55.
6. Sukumar N, Rafnsson SB, Kandala NB, Bhopal R, Yajnik CS, Saravanan P. Prevalence of vitamin B-12 insufficiency during pregnancy and its effect on offspring birth weight: a systematic review and meta-analysis. Am J Clin Nutr. 2016;103(5):1232-51. Doi: 10.3945/ajcn.115.123083. Erratum in: Am J Clin Nutr. 2017;105(1):241. PMID: 27076577..
7. Hübner U, Alwan A, Jouma M, Tabbaa M, Schorr H, Herrmann W. Low serum vitamin B12 is associated with recurrent pregnancy loss in Syrian women. Clin Chem Lab Med. 2008;46(9):1265-9. Doi: 10.1515/CCLM.2008.247. PMID: 18636794.
8. Mujawar SA, Patil VW, Daver RG. Study of serum homocysteine, folic Acid, and vitamin b(12) in patients with preeclampsia. Indian J Clin Biochem. 2011;26(3):257-60. Doi: 10.1007/s12291-011-0109-3. PMID: 22754189, PMCID: PMC3162959.
9. Pisal H, Dangat K, Randhir K, Khaire A, Mehendale S, Joshi S. Higher maternal plasma folate, vitamin B12 and homocysteine levels in women with preeclampsia. J Hum Hypertens. 2019;33(5):393-9. Doi: 10.1038/s41371-019-0164-4. PMID: 30647465.
10. Kulkarni A, Mehendale S, Pisal H, Kilari A, Dangat K, Salunkhe S, Taralekar V, Joshi S. Association of omega-3 fatty acids and homocysteine concentrations in preeclampsia. Clin Nutr. 2011;30(1):60-4. Doi: 10.1016/j.clnu.2010.07.007. PMID: 20719412.
11. Wadhwani NS, Pisal HR, Mehendale SS, Joshi SR. A prospective study of maternal fatty acids, micronutrients, and homocysteine and their association with birth outcome. Matern Child Nutr. 2015;11(4):559-73. Doi: 10.1111/mcn.12062. PMID: 23795920, PMCID: PMC6860284.
12. Mishra J, Tomar A, Puri M, Jain A, Saraswathy KN. Trends of folate, vitamin B12, and homocysteine levels in different trimesters of pregnancy and pregnancy outcomes. Am J Hum Biol. 2020;32(5):e23388. Doi: 10.1002/ajhb.23388. PMID: 31898383.
13. Muthayya S, Kurpad AV, Duggan CP, Bosch RJ, Dwarkanath P, Mhaskar A, et al. Low maternal vitamin B12 status is associated with intrauterine growth retardation in urban South Indians. Eur J Clin Nutr. 2006;60(6):791-801. Doi:10.1038/sj.ejcn.1602383. PMID: 16404414.
14. Chen X, Zhang Y, Chen H, Jiang Y, Wang Y, Wang D, et al. Association of maternal folate and vitamin B12 in early pregnancy with gestational diabetes mellitus: a prospective cohort study. Diabetes Care. 2021;44(1):217-23. Doi: 10.2337/dc20-1607. PMID: 33158950; PMCID: PMC7783943.
15. Krishnaveni GV, Hill JC, Veena SR, Bhat DS, Wills AK, Karat CL, et al. Low plasma vitamin B12 in pregnancy is associated with gestational 'diabesity' and later diabetes. Diabetologia. 2009;52(11):2350-8. Doi: 10.1007/s00125-009-1499-0. PMID: 19707742, PMCID: PMC3541499.
16. Yajnik CS, Deshpande SS, Jackson AA, Refsum H, Rao S, Fisher DJ, et al. Vitamin B12 and folate concentrations during pregnancy and insulin resistance in the offspring: the Pune Maternal Nutrition Study. Diabetologia. 2008;51(1):29-38. Doi: 10.1007/s00125-007-0793-y. PMID: 17851649, PMCID: PMC2100429.