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Research Article

Clinical, Biochemical and Hematological Response to Vitamin B12 Supplementation in Pregnant Women with Vitamin B12 Deficiency Anemia: An Observational Study

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ABSTRACT

Background: Vitamin B12 deficiency is an often overlooked cause of anemia during pregnancy, particularly in populations with vegetarian dietary practices. Limited data exist regarding its clinical and hematological response to supplementation. **Objective:** To evaluate the clinical, biochemical, and hematological response to vitamin B12 supplementation in pregnant women with vitamin B12 deficiency anemia. **Methods:** This observational study included 40 pregnant women (<36 weeks gestation) with macrocytic anemia and confirmed vitamin B12 deficiency. All participants received intramuscular vitamin B12 (1000 µg) along with oral iron and folic acid supplementation. Hematological parameters and serum vitamin B12 levels were assessed before and after treatment. Statistical analysis was performed using paired *t*-test. **Results:** The mean hemoglobin increased significantly from 8.4 ± 0.6 g/dL to 10.8 ± 0.8 g/dL ($p < 0.001$). Significant improvements were also observed in MCV, MCH, MCHC, reticulocyte count, and serum vitamin B12 levels (188.8 ± 6.7 pg/mL to 449.1 ± 32.3 pg/mL, $p < 0.001$). **Conclusion:** Vitamin B12 supplementation results in significant hematological and biochemical improvement in pregnant women with deficiency anemia. Routine screening and timely intervention may improve maternal and fetal outcomes.

Keywords

Vitamin B12 deficiency, Pregnancy, Macrocytic anemia, Hematological response, Antenatal care

INTRODUCTION

Anemia during pregnancy remains a major global public health concern, contributing significantly to maternal and perinatal morbidity and mortality. The most common forms include microcytic hypochromic anemia, typically associated with iron deficiency, and dimorphic anemia, characterized by the coexistence of two distinct red blood cell populations.[1,2]

Pregnancy is associated with various physiological changes in hematological parameters, including alterations in white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin concentration, packed cell volume, and platelet count. These changes collectively contribute to what is

termed the "physiological anemia of pregnancy."[3] This condition results primarily from hemodilution due to an increase in plasma volume exceeding the rise in red cell mass. Physiological anemia is considered beneficial as it reduces blood viscosity, enhances vascular perfusion, and ensures adequate oxygen and nutrient delivery to the developing fetus.[4]

Optimal maternal nutrition plays a crucial role in ensuring healthy fetal development throughout pregnancy.[5] Among the various regulators of iron metabolism, hepcidin—a hormone synthesized in the liver—plays a key role in controlling iron absorption and recycling. Hepcidin levels increase in response to inflammation and iron overload, while they decrease during hypoxia and increased erythropoietic activity.[6] The nutritional status of a pregnant woman significantly influences both maternal health and fetal outcomes.[7]

Vitamin deficiencies, particularly vitamin D and vitamin B12, are common during pregnancy and can have substantial clinical implications. Vitamin D deficiency is widespread and may adversely affect maternal and fetal health, increasing the risk of adverse perinatal outcomes. Hemodilution during pregnancy may lead to reduced plasma concentrations of certain vitamins, although some remain stable due to increased carrier proteins.[8,9] Vitamin B12 is essential for proper folate metabolism and DNA synthesis, both of which are critical for cell proliferation during pregnancy. Deficiency of vitamin B12 is often linked to inadequate dietary intake, increased maternal metabolic demands, and active placental transfer to the fetus. This can lead to decreased maternal B12 levels and, consequently, low levels in the newborn.[10]

Both vitamin B12 and folic acid are vital for DNA synthesis and red blood cell maturation. Deficiency of either nutrient results in impaired nuclear maturation with relatively normal cytoplasmic development, leading to macrocytic anemia. This condition is characterized by ineffective erythropoiesis, intramedullary hemolysis, pancytopenia, and characteristic morphological abnormalities in blood and bone marrow cells.[11] Vitamin B12 deficiency during pregnancy is associated with several adverse reproductive outcomes, including recurrent abortions, infertility, and preterm labor.[12] In infants, it may lead to growth retardation, delayed psychomotor development, neurological abnormalities, and long-term neurodevelopmental deficits.[13]

Although oral vitamin B12 supplementation provides a convenient and cost-effective alternative to parenteral therapy, its efficacy may be limited due to variable absorption.[14] Therefore, parenteral administration is often considered more effective. However, there is currently no consensus regarding the optimal dosage, duration, or route of vitamin B12 supplementation in pregnant women with anemia. While extensive research exists on iron deficiency anemia in pregnancy, studies focusing specifically on vitamin B12 deficiency anemia remain limited. Hence, the present study aims to evaluate the clinical, biochemical, and hematological response to vitamin B12 supplementation in pregnant women diagnosed with vitamin B12 deficiency anemia

METHODS

Study Design and Setting

This observational study was conducted in the Department of Obstetrics and Gynaecology at GMC, Doda, Jammu and Kashmir, India.

Study Population

A total of 75 pregnant women with macrocytic anemia were initially

screened for vitamin B12 deficiency using serum vitamin B12 estimation. Among them, 40 pregnant women diagnosed with vitamin B12 deficiency were enrolled in the study.

Inclusion Criteria

Pregnant women were included if they met the following criteria:

- Singleton pregnancy with gestational age <36 weeks
- Hemoglobin level <10.0 g/dL
- Mean corpuscular volume (MCV) >100 fL
- Platelet count >100,000 cells/mm³
- No history of recent blood transfusion
- Absence of significant comorbidities or known hemoglobinopathies

Intervention

All enrolled participants received intramuscular vitamin B12 at a dose of 1000 µg. In addition, all patients were administered routine antenatal supplementation with oral iron (200 mg/day) and folic acid (500 µg/day).

Sample Collection and Laboratory Analysis

A total of 10 mL of venous blood was collected under aseptic conditions, both with and without anticoagulant, for plasma and serum separation. Samples were centrifuged at 2500 rpm for 10 minutes, and plasma and serum were stored at −80°C until analysis.

Additionally, 2 mL of whole blood was collected in a dipotassium EDTA vial for hematological analysis. A peripheral blood smear was prepared simultaneously to assess red cell morphology and classify the type of anemia.

Hematological parameters, including hemoglobin (Hb), total leukocyte count (TLC), platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and reticulocyte count, were analyzed using an automated hematology analyzer (Sysmex KX-21, three-part differential).

Serum vitamin B12 levels were measured using a chemiluminescent enzyme immunoassay (IMMULITE/IMMULITE 1000 Vitamin B12 system).

Follow-up and Outcome Measures

Patients were followed up to assess hematological and biochemical response to treatment. Reticulocyte count was repeated at 7–10 days, while hemoglobin levels were assessed at 10–14 days and again at 25–30 days post-treatment. Complete blood count and serum vitamin B12 levels were re-evaluated at 25–30 days, with an additional vitamin B12 estimation at 7–10 days.

The primary outcome measures included changes in serum vitamin B12 levels, hemoglobin concentration, total leukocyte count, platelet count, reticulocyte count, and red blood cell indices before and after treatment.

Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 24. Continuous variables were expressed as mean ± standard deviation. Pre- and post-treatment values were compared using the paired *t*-test. A *p*-value of <0.05 was considered statistically significant.

RESULTS

Demographic Profile of the Study Population

A total of 40 pregnant women with vitamin B12 deficiency anemia were included in the study. The mean age of the participants was 28.5 ± 3.3 years (range: 19–33 years). The majority of the women were multigravidae (85%), followed a vegetarian diet (82.5%), and belonged to a lower socioeconomic status (62.5%).

The mean gestational age at presentation was 33 ± 2 weeks (range: 29+3 to 33+6 weeks). Fatigue was the most common presenting complaint (70%), followed by breathlessness (27.5%), loss of appetite (17.5%),

passage of worms in stools (17.5%), pica (12.5%), palpitations (10%), and history of bleeding (2.5%). Several participants reported more than one symptom.

Table 1: Demographic and Clinical Profile of Patients (n = 40)

Demographic profile	Number of cases (n=60)	Percebtage (%)
Age (years)	28.5±3.3	
Multigravida	34	85
Vegetarian	33	82.5
lower socio-economic status	25	62.5
gestational age	33±2	
Fatigue	28	70
Breathlessness	11	27.5
loss of appetite	7	17.5
passage of worms in stools	7	17.5
Pica	5	12.5
Palpitation	4	10
History of bleeding	1	2.5

Effect of Treatment

Comparison of hematological parameters and serum vitamin B12 levels before and after treatment demonstrated a statistically significant improvement in all measured variables ($p < 0.05$). Hemoglobin levels increased significantly from 8.4 ± 0.6 g/dL to 10.8 ± 0.8 g/dL following treatment. A significant rise in total leukocyte count and reticulocyte count was also observed, indicating an appropriate bone marrow response. Mean corpuscular volume (MCV) showed a significant reduction from 101.8 ± 4.65 fL to 95.5 ± 2.79 fL, reflecting improvement in macrocytosis. Similarly, MCH and MCHC values demonstrated statistically significant changes post-treatment. Serum vitamin B12 levels increased markedly from 188.8 ± 6.7 pg/mL to 449.1 ± 32.3 pg/mL, confirming the biochemical effectiveness of supplementation

Table 2: Comparison of Hematological Parameters Before and After Treatment (n = 40)

Parameter	Pretreatment Mean ± SD	Post treatment Mean ± SD	P-value
Hb (gm/dl)	8.4±0.6	10.8±0.8	0.000
TLC (cells/cumm)	5443.4±163.8	7713.4±678.4	0.000

Platelet count (lac cells/cumm)	1.94±0.38	1.37±0.21	0.000
MCV (fl)	101.8±4.65	95.5±2.79	0.000
MCH (pg)	32.75±4.8	29.5±4.4	0.002
MCHC (gm/dl)	32.05±4.26	30.5±4.4	0.0115
Reticulocyte count	0.57±0.11	0.79±0.08	0.000
Serum Vit B12	188.8±6.7	449.1±32.3	0.000

DISCUSSION

Anemia during pregnancy continues to be a major global public health concern, with significant implications for both maternal and fetal outcomes. Although iron deficiency remains the most common cause, the contribution of other nutritional deficiencies, particularly vitamin B12 deficiency, is increasingly recognized.

Demographic Profile

In the present study, the mean age of participants was 28.5 ± 3.3 years, which corresponds to the peak reproductive age group. This is consistent with the higher incidence of pregnancy and associated complications, including anemia, in women aged 20–30 years. The majority of participants were multigravidae, highlighting the association between multiparity and anemia. Repeated pregnancies, especially with inadequate interpregnancy intervals, may lead to depletion of essential nutrients such as iron, folic acid, and vitamin B12, thereby increasing the risk of anemia.

A significant proportion of women in the study belonged to lower socioeconomic strata, which is known to influence nutritional status, access to healthcare, and overall pregnancy outcomes. Additionally, 82.5% of the participants followed a vegetarian diet, which is an important risk factor for vitamin B12 deficiency, as this vitamin is predominantly found in animal-based foods. Previous studies have also reported a higher prevalence of vitamin B12 deficiency among vegetarians and vegans.[15]

Hematological Response to Treatment

All participants in the present study had moderate anemia at enrollment, with a mean hemoglobin level of 8.4 ± 0.6 g/dL. Following vitamin B12 supplementation, a significant improvement in hemoglobin levels was observed, rising to 10.8 ± 0.8 g/dL by 25–30 days. This improvement was statistically significant ($p < 0.001$) and was accompanied by a reduction in the severity of anemia.

These findings are consistent with earlier studies, such as that by Adams et al., which demonstrated a favorable hematological response to vitamin B12 therapy.[16] The observed improvement can be attributed to the essential role of vitamin B12 in DNA synthesis and erythropoiesis. Deficiency of vitamin B12 leads to defective nuclear maturation, resulting in nuclear–cytoplasmic asynchrony, ineffective erythropoiesis, and intramedullary destruction of erythroid precursors. Correction of this deficiency restores effective hematopoiesis, thereby improving hemoglobin levels.[17]

A significant increase in total leukocyte count and reticulocyte count was also noted, indicating an appropriate bone marrow response to therapy. Reticulocytosis is an early indicator of

effective treatment and reflects recovery of erythropoietic activity.

Changes in Red Cell Indices

The mean corpuscular volume (MCV) showed a significant reduction from 101.8 ± 4.65 fL to 95.5 ± 2.79 fL following treatment, indicating resolution of macrocytosis. Elevated MCV is a hallmark of vitamin B12 deficiency and results from impaired DNA synthesis leading to the production of large, immature erythrocytes.

Similarly, mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) also demonstrated statistically significant changes after treatment. These findings further support the correction of ineffective erythropoiesis following vitamin B12 supplementation.

Biochemical Response

Serum vitamin B12 levels increased significantly from 188.8 ± 6.7 pg/mL to 449.1 ± 32.3 pg/mL ($p < 0.001$), confirming the biochemical efficacy of supplementation. These levels fall within the normal reference range (200–900 pg/mL), indicating adequate replenishment of vitamin stores. The observed improvement is consistent with the known pharmacokinetics of vitamin B12, which has a biological half-life of approximately 6 days and is stored in the liver for extended periods. Studies have suggested that a single intramuscular dose of 1000 µg may be sufficient to meet the increased physiological demands during pregnancy.[22] During pregnancy, especially in the third trimester, serum vitamin B12 levels tend to decline due to increased maternal and fetal requirements, as well as hemodilution.[23] This underscores the importance of timely diagnosis and supplementation.

Clinical Implications and Treatment Considerations

Vitamin B12 plays a critical role in maternal and fetal health, and its deficiency is associated with adverse outcomes such as preterm birth, low birth weight, and neurodevelopmental impairment in infants. Although oral vitamin B12 supplementation is convenient and cost-effective, its absorption may be unpredictable, particularly in individuals with malabsorption or severe deficiency. In contrast, intramuscular administration ensures reliable absorption and rapid correction of deficiency. Alternative routes such as sublingual and intranasal preparations are available but are less commonly used due to higher costs and limited evidence in pregnancy. Despite the growing recognition of vitamin B12 deficiency in pregnancy, there is a lack of standardized guidelines regarding the optimal dosage, duration, and route of administration. Most existing research has focused on iron deficiency anemia, leaving a gap in evidence-based management of vitamin B12 deficiency in pregnant women.

Strengths and Limitations

The present study highlights the significant clinical and hematological improvement following vitamin B12 supplementation in pregnant women with deficiency anemia. However, the study is limited by its relatively small sample size and observational design. Additionally, the lack of a control group and long-term follow-up restricts the generalizability of the findings.

CONCLUSION

Vitamin B12 deficiency is a significant but often overlooked cause of anemia in pregnancy. This study demonstrates that vitamin B12 supplementation leads to significant improvement in hemoglobin levels, red cell indices, and serum vitamin B12 levels. Early detection and timely treatment, especially in high-risk groups, are essential. Incorporating vitamin B12 screening into routine antenatal care can help improve maternal and fetal outcomes.

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