Effects of Vitamin B12 Supplementation on some hematological parameters in Patients with Anemia due to B12 deficiency

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Abstract

Background: *Anemia is a widespread hematological disorder characterized by reduced red blood cell (RBC) count, hemoglobin concentration, or both. Among its various etiologies, vitamin B12 deficiency plays a significant role, especially in populations with dietary restrictions or malabsorption syndromes. This study evaluates the effects of vitamin B12 supplementation on RBC count and other hematological parameters in patients diagnosed with anemia due to confirmed B12 deficiency.*

Methods *:A prospective cohort of 200 patients was analyzed over 18 months, with RBC counts and hemoglobin levels measured at baseline, 6 weeks, and 12 weeks post-supplementation of intramuscular injections hydroxocobalamin, 1000 µg.*

Results*: The mean age was 45.3 ± 12.4 years, with a female predominance (60%), at baseline, the mean RBC count was 3.1 ± 0.5 × 10^12/L, which rose significantly to 3.8 ± 0.4 × 10^12/L at 6 weeks and 4.5 ± 0.3 × 10^12/L by 12 weeks (p<0.001). Hemoglobin levels increasing from 9.2 ± 1.8 g/dL to 11.5 ± 1.2 g/dL at 6 weeks and 13.0 ± 1.0 g/dL at 12 weeks (p<0.001).Mean corpuscular volume (MCV) of 110.2 ± 6.8 fL at baseline, normalized to 101.6 ± 5.9 fL at 6 weeks and 95.4 ± 5.3 fL by 12 weeks (p<0.001).*

Conclusion: *Vitamin B12 supplementation is a corner stone in managing anemia due to B12 deficiency. Prompt diagnosis and treatment significantly improve RBC count, hemoglobin levels, and patient-reported outcomes.*

Introduction

Anemia, a condition characterized by insufficient red blood cell production or function, affects over one-third of the global population and has substantial socioeconomic and health burdens (1). It can result from diverse causes, including nutritional deficiencies, chronic diseases, and genetic disorders. Among nutritional causes, vitamin B12 deficiency is particularly notable due to its association with megaloblastic anemia and neurological complications (2).

Vitamin B12, or cobalamin, is vital for DNA synthesis, particularly in rapidly dividing cells like erythroid progenitors in the bone marrow (3). Deficiency disrupts this process, leading to ineffective erythropoiesis and macrocytosis. Common causes of deficiency include dietary insufficiency—particularly among vegetarians and vegans—pernicious anemia,

gastrointestinal disorders such as celiac disease or Crohn's disease, and prolonged use of certain medications like proton pump inhibitors or metformin (4).

Symptoms of vitamin B12 deficiency are varied, ranging from generalized fatigue and pallor to more severe neurological manifestations, including paresthesia, ataxia, and cognitive impairment. The hematological effects, however, are among the most clinically recognized, with megaloblastic anemia being a hallmark (5). Early diagnosis and intervention are crucial to prevent irreversible complications, yet diagnosis can be challenging due to overlapping symptoms with other conditions and the variability of serum B12 levels (6).

Recent advances in diagnostic methodologies, such as serum methylmalonic acid and homocysteine as adjunct markers, have improved the accuracy of diagnosing B12 deficiency (7). Additionally, the growing understanding of the global prevalence of dietary insufficiency highlights the importance of public health initiatives to address vitamin B12 deficiency in at-risk populations (8,9).

This study aims to investigate the efficacy of vitamin B12 supplementation in improving hematological parameters, specifically RBC count and hemoglobin levels, in patients with anemia due to confirmed B12 deficiency. By delineating the patterns of hematological recovery, the findings could provide valuable insights for clinicians in tailoring management strategies.

Patients and Methods

Study Design and patients

Prospective cohort study was conducted at a tertiary care hospital in Babylon province from April 2023 to October 2024 . A total of 200 patients aged 18–65 years diagnosed with anemia due to confirmed vitamin B12 deficiency were enrolled. Exclusion criteria included patients with anemia from other causes (iron deficiency or hemoglobinopathies), also patients with recent blood transfusion, or coexisting severe chronic illnesses are excluded.

Diagnostic Criteria

Anemia was defined according to World Health Organization (WHO) standards: hemoglobin levels <13 g/dL in men and <12 g/dL in women (10). Vitamin B12 deficiency was confirmed with serum levels <200 pg/mL.(11) Additional diagnostic evaluations were done including serum methylmalonic acid and homocysteine levels.

Methods

1. Vitamin B12 Measurement: Vitamin B12 levels were measured using a chemiluminescent immunoassay (CLIA) on an automated analyzer. The assay relies on the competitive binding principle, where labeled cobalamin competes with the patient's serum B12 for binding to intrinsic factor-coated magnetic particles. After washing, chemiluminescent signals

proportional to the B12 concentration were detected. Results below 200 pg/mL were considered deficient.

2. Methylmalonic Acid (MMA): MMA levels were quantified using gas chromatography-mass spectrometry (GC-MS). Serum samples were first extracted and derivatized to produce methylmalonyl derivatives. The GC-MS system detected MMA levels with high sensitivity and specificity. Elevated MMA (>0.4 µmol/L) was considered indicative of functional vitamin B12 deficiency.

3. Homocysteine: Total homocysteine levels were measured using high-performance liquid chromatography (HPLC) with fluorescence detection. Serum homocysteine was reduced and derivatized before separation by HPLC. Concentrations above 15 µmol/L were considered elevated, suggestive of disrupted methionine metabolism due to B12 deficiency.

4. **CBC:** This was done by using autoanalyzer (Abacus Diatron company).

Intervention

Patients received intramuscular injections of hydroxocobalamin, 1000 µg (to provide good therapeutic response in those with malabsorption and sever B12 deficiency) , administered weekly for the first 4 weeks, followed by monthly injections. Compliance was monitored through hospital records.

Outcome Measures

Primary outcomes were changes in RBC count and hemoglobin levels, measured at baseline, 6 weeks, and 12 weeks post-supplementation. Secondary outcomes included mean corpuscular volume (MCV) and patient-reported symptoms.

Statistical Analysis

Data were analyzed using SPSS software (version 26). Continuous variables were expressed as mean ± standard deviation (SD), and categorical variables as frequencies and percentages. Paired t-tests and repeated-measures ANOVA were used to compare baseline and post-intervention values. A p-value <0.05 was considered statistically significant.

Results

Baseline Characteristics

The cohort's mean age was 45.3 ± 12.4 years, with a female predominance (60%). Common symptoms at presentation included fatigue (90%), pallor (85%), and glossitis (40%). Mean baseline hemoglobin and RBC count were 9.2 ± 1.8 g/dL and 3.1 ± 0.5 × 10^12/L, respectively.

Hematological Response to Vitamin B12 Supplementation

Hematological Parameters(Table1):

Patients exhibited significant hematological recovery post-vitamin B12 supplementation. At baseline, the mean RBC count was 3.1 ± 0.5 × 10^12/L, which rose significantly to 3.8 ± 0.4 × 10^12/L at 6 weeks and 4.5 ± 0.3 × 10^12/L by 12 weeks ($p<0.001$). Hemoglobin levels increasing from 9.2 ± 1.8 g/dL to 11.5 ± 1.2 g/dL at 6 weeks and 13.0 ± 1.0 g/dL at 12 weeks ($p<0.001$). Additionally, macrocytosis, indicated by an elevated mean corpuscular volume (MCV) of 110.2 ± 6.8 fL at baseline, normalized to 101.6 ± 5.9 fL at 6 weeks and 95.4 ± 5.3 fL by 12 weeks ($p<0.001$).

Table 1. Hematological Parameters at Baseline, 6 Weeks, and 12 Weeks

Parameter	Baseline	6 Weeks	12 Weeks	p-value
RBC Count (×10^12/L)	3.1 ± 0.5	3.8 ± 0.4	4.5 ± 0.3	<0.001
Hemoglobin (g/dL)	9.2 ± 1.8	11.5 ± 1.2	13.0 ± 1.0	<0.001
MCV (fL)	110.2 ± 6.8	101.6 ± 5.9	95.4 ± 5.3	<0.001

Symptom Resolution (Table 2):

Clinical symptoms associated with vitamin B12 deficiency showed marked improvement. Fatigue, reported by 90% of patients at baseline, decreased to 50% at 6 weeks and 20% by 12 weeks. Pallor showed similar improvements, reducing from 85% at baseline to 55% at 6 weeks and 25% at 12 weeks. Glossitis, present in 40% of patients initially, was resolved in most cases by 12 weeks (10%).

Table 2. Symptoms Resolution Over Time

Symptom	Baseline (%)	6 Weeks (%)	12 Weeks (%)
Fatigue	90	50	20
Pallor	85	55	25
Glossitis	40	20	10

Biochemical Markers (Table 3):

Vitamin B12 supplementation led to significant improvements in serum B12 and related biomarkers. Serum B12 levels increased from 150 ± 30 pg/mL at baseline to 450 ± 50 pg/mL at 12 weeks ($p<0.001$). Similarly, methylmalonic acid levels, elevated at baseline (0.56 ± 0.15 µmol/L), normalized to 0.22 ± 0.08 µmol/L by 12 weeks ($p<0.001$). Homocysteine levels, a marker of impaired methionine metabolism, decreased from 19.4 ± 4.2 µmol/L at baseline to 9.1 ± 2.5 µmol/L at 12 weeks ($p<0.001$).

Table 3. Serum Biomarkers of Vitamin B12 Deficiency

Biomarker	Baseline	12 Weeks	p-value
Serum B12 (pg/mL)	150 ± 30	450 ± 50	<0.001
Methylmalonic Acid (µmol/L)	0.56 ± 0.15	0.22 ± 0.08	<0.001
Homocysteine (µmol/L)	19.4 ± 4.2	9.1 ± 2.5	<0.001

Discussion

Vitamin B12 supplementation effectively increased RBC count and corrected hematological abnormalities in patients with B12 deficiency anemia. The observed timeline of recovery aligns with the physiological role of B12 in erythropoiesis and DNA synthesis (12).

According to our results, the majority of study participants were middle aged and older with female preponderance ,this finding is not consistent with study from India , where vitamin B12 insufficiency was prevalent in male(2) size of the sample may explain this differences.

The significant improvements in RBC count, hemoglobin levels, and normalization of MCV highlight the efficacy of B12 supplementation. These findings support its role as a corner stone therapy for megaloblastic anemia, which are concordant with previous studies(13,14) . Additionally, the rapid resolution of symptoms such as fatigue and pallor underscores the clinical benefits of timely intervention (15).

Substantial improvement was also reflected in biochemical markers. The normalization of methylmalonic acid and homocysteine levels supports the biochemical efficacy of supplementation, as both markers are sensitive indicators of cellular B12 activity (16). This is particularly important in differentiating B12 deficiency from other causes of anemia and ensuring appropriate treatment (17).Numerous studies have examined the relationship between vitamin B12 and homocysteine ,Premkumar et al. found that treating B12 deficiency lowers homocysteine level and reducing the risk of thrombotic events (18).

Despite these positive outcomes, challenges remain. For instance, specific subgroups, such as older adults and individuals with chronic gastrointestinal disorders, may experience delayed or incomplete hematological recovery, necessitating prolonged or higher-dose supplementation (19). Personalized treatment strategies considering comorbidities and baseline biochemical profiles could enhance patient outcomes (20,21).

Conclusion

Vitamin B12 supplementation is a corner stone in managing anemia due to B12 deficiency. Prompt diagnosis and treatment significantly improve RBC count, hemoglobin levels, and patient-reported outcomes. Future studies should explore the long-term impact of B12 supplementation on neurological outcomes, as this aspect remains under-researched despite its critical implications. Additionally, large-scale public health initiatives could address the rising prevalence of dietary B12 deficiency, particularly in populations adhering to plant-based diets

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