



RESEARCH ARTICLE

STUDY OF BIOCHEMICAL AND HAEMATOLOGICAL RESPONSE OF VITAMIN B12
SUPPLEMENTATION IN PREGNANT ANAEMIC WOMEN WITH
VITAMIN B12 DEFICIENCY

^{1,*}Dr. Roopali Goyal Gandhi, ²Dr. Manju Puri, ³Dr. Shailaja Shukla and ⁴Dr. Amita Yadav

^{1,2}Department of Obstetrics and Gynaecology, Lady Hardinge Medical College, New Delhi, India

³Department of Pathology, Lady Hardinge Medical College, New Delhi, India

⁴Department of Biochemistry, Lady Hardinge Medical College, New Delhi, India

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ABSTRACT

Background: There are limited studies on biochemical and haematological response to vitamin B12 supplementation in pregnant women with vitamin B12 deficiency in literature.

Aim: To evaluate the biochemical and haematological response of vitamin B12 supplementation in pregnant women with vitamin B12 deficiency anaemia.

Method: Thirty pregnant women with macrocytic anaemia due to vitamin B12 deficiency with gestation <36 weeks, hemoglobin <10.0 gm/dl, MCV > 100 fl, platelet count >30,000 cells/mm³ with no other co-morbidity were administered a single dose of 1000 µg of vitamin B12 intramuscularly. Blood samples were subjected to complete blood count with peripheral smear, reticulocyte count and vitamin B12 levels before and 4 weeks after vitamin B12 administration.

Results: Majority (90%) were multigravidae and 90% were vegetarian. Fatigue was the commonest complaint (70%). Mean hemoglobin improved from 8.76 ± 0.65 to 10.53 ± 0.63 gm/dl, TLC increased from 5483.33 ± 866.65 cells/cumm to 7726.66 ± 1275.75 cells/cumm, platelet count improved from 1.86 ± 0.44 lac cells/cumm to 3.16 ± 0.66 lac cells/cumm, MCV decreased from 102.1 ± 1.6 fl to 96.4 ± 1.55 fl. Mean MCH decreased from 32.87 ± 0.65 pg to 29.39 ± 0.7 pg. MCHC changed from 32.18 ± 0.85 gm/dl to 30.47 ± 0.84 gm/dl, Reticulocyte count increased from $0.52 \pm 0.14\%$ to $0.78 \pm 0.18\%$ and vitamin B12 levels improved from 189.83 ± 10.85 pg/ml. to 435 ± 107.91 pg/ml. The difference was statistically significant, $P < 0.001$ for all values.

Conclusion: A single dose of 1000 µg vitamin B12 intramuscular administration results in a statistically significant increase in serum vitamin B12 levels in pregnant women with vitamin B12 deficiency anaemia.

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INTRODUCTION

Anaemia is the most common nutritional deficiency disorder in the world. Anaemia affects almost two-thirds of pregnant women in developing countries and contributes to maternal morbidity and mortality and low birth weight (Baig et al., 2008). Nutritional anaemia gets aggravated consequent to pregnancy because of increased requirement of iron, folic acid and vitamin B12. Iron and folic acid supplementation is routinely advocated in pregnancy; however vitamin B12 supplementation is not a part of routine antenatal care (Pathak et al., 2007). Vitamin B12 is derived from non-vegetarian food. Most women in India are vegetarian; hence its deficiency is common.

*Corresponding author: Dr. Roopali Goyal,

Department of Obstetrics and Gynaecology, Lady Hardinge Medical College, New Delhi, India.

Vitamin B12 and folic acid are essential for DNA synthesis. Deficiency of either results in asynchrony in the maturation of the nucleus and cytoplasm of rapidly regenerating red cells and manifests as macrocytic anaemia. In the haematopoietic system this asynchrony results in abnormal nuclear maturation with normal cytoplasmic maturation, intramedullary haemolysis, ineffective erythropoiesis, pancytopenia and typical morphological abnormalities in blood and bonemarrow cells (Khanduri and Sharma, 2007; Antony, 2005). Low level of vitamin B12 affects reproduction and can result in recurrent abortions, infertility and preterm labour among pregnant women (Molloy et al., 2008). It may cause growth retardation, delayed psychomotor development, abnormal movements and permanent effects on developing brain in infants (KOC et al., 2006; Cagnacci et al., 2004; Doshi et al., 2006; Schneede et al., 1994). The oral administration of vitamin B12 offers a potentially simpler and cheaper alternative to parenteral

administration, but its effectiveness is affected by erratic absorption (Sanz *et al.*, 2012). Hence parenteral administration is considered to be a superior mode of administration. However, there is no consensus on the optimum dosage, duration and the route of administration of vitamin B12 in pregnant anaemic women. The literature is replete with studies on iron deficiency anaemia during pregnancy. However there are very few studies on vitamin B12 deficiency causing anaemia during pregnancy. Our study aims to evaluate the clinical, biochemical and haematological response of vitamin B12 supplementation in pregnant women with vitamin B12 deficiency anaemia.

METHODS

This observational study was conducted in the Department of Obstetrics and Gynaecology, Lady Hardinge Medical College, New Delhi from December 2013 to March 2015. Seventy-two pregnant women with macrocytic anaemia fulfilling the selection criteria were screened for vitamin B12 deficiency by serum vitamin B12 estimation. Of these 30 pregnant women with vitamin B12 deficiency were enrolled in the study. These pregnant women were administered vitamin B12 (1000 µg intramuscularly), in addition to supplements of iron and folic acid in the dosage of 200 mg and 500 µg per day respectively as per routine antenatal care.

Selection and description of participants

Thirty pregnant women with singleton pregnancy at <36 weeks of gestation, Haemoglobin <10.0 gm/dl, mean corpuscular volume (MCV) > 100 fl, Platelet >30,000 cells/mm³, no history of blood transfusion, co-morbidity and haemoglobinopathies with macrocytic anaemia were included in the study.

Technical information

Ten ml of venous blood sample with and without anticoagulant was drawn for plasma and serum respectively.

The patients were subjected to a repeat reticulocyte count between 7-10 days, haemoglobin levels between 10-14 days and 25-30 days and complete blood count between 25-30 days post treatment. Serum vitamin B12 levels were estimated between 7-10 days and 25-30 days post treatment. The outcome variables in this study included change in pre and post treatment values of serum vitamin B12 levels, haemoglobin levels, TLC, platelet count, reticulocyte count and RBC indices.

Statistical analysis

The analysis was done using Statistical Package for the Social Sciences (SPSS) version 17. Pretreatment and post treatment parameters within the group were compared using paired t test. Statistical significance was taken as p value < 0.05.

RESULTS

Demographic profile of the patients

The mean age of the pregnant women included in the study was 26 ± 3.74 years with a range of 19-33 years. Ninety percent of the women were multigravidae, 93% were vegetarian and 53.33% patients belonged to lower socio-economic status. The mean gestational age was 33 ± 2 weeks ranging from 29+3 to 33+6 weeks. Fatigue was the commonest complaint (70%) whereas breathlessness was present in 26.66 %, loss of appetite in 16.66 %, passage of worms in stools in 16.66%, pica in 13.33 %, palpitations in 10 % and history of bleeding from any site in 3.33 %. Some women had more than one symptom.

Effect of treatment

Table no. 1 shows the comparison of values of various haematological parameters and serum vitamin B12 before and after treatment. All parameters studied showed a statistically significant improvement (P < 0.05).

Table 1

Parameter	Pretreatment		Post treatment		Diff in the means	p value
	Mean ± SD	Range	Mean ± SD	Range		
Hb (gm/dl)	8.76 ± 0.65	7.6 - 9.8	10.53 ± 0.63	9.2 - 11.6	1.77	<0.001
TLC (cells/cumm)	5483.33 ± 866.65	4100 - 7200	7726.66 ± 1275.75	5100 - 10100	2243.33	<0.001
Platelet count (lac cells/cumm)	1.86 ± 0.44	1.3 - 2.7	3.16 ± 0.66	2.1 - 4.5	1.3	<0.001
MCV (fl)	102.1 ± 1.6	100 - 106.6	96.4 ± 1.55	93.1 - 99.8	5.72	<0.001
MCH (pg)	32.87 ± 0.65	31.8 - 34.6	29.39 ± 0.7	28.1 - 30.5	3.48	<0.003
MCHC (gm/dl)	32.18 ± 0.85	30.3 - 34.1	30.47 ± 0.84	28.9 - 31.9	1.76	<0.001
Reticulocyte count	0.52 ± 0.14	0.3 - 0.8	0.78 ± 0.18	0.5 - 1.2	0.28	<0.001
Serum Vit B12	189.83 ± 10.05	162 - 199	435 ± 107.92	252 - 684	245.17	< 0.001

Plasma and sera were separated by centrifugation at 2500 rpm for 10 minutes and stored at -80° C. Two ml of whole blood was collected in a di-potassium EDTA coated vial after performing a clean venous puncture. At the same time a peripheral smear was prepared to look for red cell morphology and type of anemia. All the samples were analyzed for haemoglobin (Hb), total leukocyte count (TLC), platelet count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and Reticulocyte count using haematology auto analyzer system (Sysmex KX-21 three part cell coulter). Serum levels of vitamin B12 were measured by chemiluminescent enzyme immunoassay using IMMULITE/IMMULITE 1000 Vitamin B12.

DISCUSSION

Demographic profile

The mean age of the women studied was 26 ± 3.74 years. The age group of 20-30 years is a high fertility age group and the majority of conceptions occur in this age group hence a higher incidence of obstetric complications including anaemia. In our study majority of the women were multigravidae. Repeated childbirth and abortions lead to anaemia, hence multiparity is associated with anaemia. With frequent pregnancies and lack of spacing between births, replenishment of nutrients like iron, folic acid and vitamin B12 is inadequate, increasing the chances of anemia. Literacy and socio-economic status are

interlinked and also influence the prevalence of anaemia in pregnant women. The socio-economic development, higher standard of living, better utilization of health care facilities, along with increasing literacy rate, are associated with the low prevalence of anaemia in developed countries. Most of the pregnant women in the study population (93%) were vegetarian. Vegetarian food is deficient in vitamin B12 hence individuals following vegetarian diet are at risk for developing vitamin B12 deficiency. Higher prevalence of vitamin B12 deficiency has been reported in vegans as compared to other vegetarians (Pawlak *et al.*, 2014; Gatenby *et al.*, 1960).

Haematological profile

In our study all patients had moderate anaemia at enrollment. Hb levels ranged from 7.6 to 9.8 gm/dl, mean being 8.76 ± 0.65 gm/dl. Post treatment hemoglobin was checked twice, between day 10-14 and day 25-30. On first check, the mean Hb levels increased to 9.47 ± 0.75 gm/dl. Mild anaemia was found in 33.3% and moderate anaemia in 66.6%. On second check, mean Hb levels rose to 10.53 ± 0.63 gm/dl. Twenty percent of women had normal Hb, 56.6% had mild anaemia and only 23.3% had moderate anaemia. Hence on both assessments, there was rise in Hb levels and reduction in severity of anaemia. The difference was found to be statistically significant (p value < 0.001). Adams *et al.* (1952) studied the response to 100 µg of vitamin B12 injections given for 10 days (1000 µg total dose). The mean pretreatment hemoglobin was 6.0 gm/dl and mean post treatment (after two weeks) Hb was 7.7 gm/dl, indicating a good response to the treatment (Adams, 1956). Vitamin B12 and folic acid are coenzymes required for the synthesis of thymidine, one of the four bases found in DNA. Defective DNA synthesis results in impaired nuclear development while the cytoplasmic maturation proceeds normally as synthesis of RNA and protein is relatively unaffected. This gives rise to nuclear cytoplasmic asynchrony.

The erythroblasts become large, oval shaped. These megaloblast precursors do not mature enough to be released into the circulation. They undergo intramedullary destruction, hence anaemia develops. Treatment with vitamin B12 increases hematopoiesis hence total leucocyte count and platelet count increases (Antony, 2012; Krukow, 2008). In our study, pretreatment mean MCV mean was 102.1 ± 1.6 fl and post treatment mean MCV was 96.4 ± 1.55 fl. The difference was found to be statistically significant (p value < 0.001). Mean corpuscular volume (MCV) is the average volume of red cells. Normal MCV values range from 79 to 93 femtoliters (fl), (Kratz *et al.*, 2008; Roberts *et al.*, 1963), $MCV > 100$ fl is a sign of macrocytosis. Macrocytosis due to vitamin B12 is a direct result of ineffective or dysplastic erythropoiesis. Due to nuclear cytoplasmic asynchrony, the erythroblasts become large, oval shaped with resultant increase in MCV (Kratz *et al.*, 2008; Roberts *et al.*, 1963). Hence treatment with vitamin B12 decreases MCV. The mean corpuscular haemoglobin (MCH) is the average mass of hemoglobin per red blood cell, normal values ranging from 27 to 33 pg/cell in adults (Chanarin, 1979). The mean corpuscular haemoglobin concentration (MCHC) is a measure of the concentration of Hb in a given volume of packed red blood cell, normal values ranging from 33 to 36 g/dl respectively in adults (Chanarin, 1979). In our study, pretreatment mean MCH mean was 32.87 ± 0.65 pg and post treatment mean was 29.39 ± 0.7 pg. The difference was found to be statistically significant (p value < 0.003) possibly indicating an earlier response in terms of decrease in MCV

compared to MCH. Pretreatment mean MCHC was 32.18 ± 0.85 gm/dl and post-treatment mean MCHC was 30.47 ± 0.84 gm/dl. The difference was found to be significant (p value < 0.001). Replacement therapy with the erythropoietic factors results in an increase in the reticulocyte count. Hence a statistically significant increase in reticulocyte count was observed after Vitamin B12 supplementation.

Biochemical parameter

Serum Vitamin B12

Normal values of serum vitamin B12 are 200 - 900 pg/ml (James *et al.*, 2011). Fasting level of less than 200pg/ml is suggestive of deficiency of vitamin B12 (Pardo *et al.*, 2004). In view of biological half-life of vitamin B12 of 6 days and long duration of storage in liver for 400 days, McLintock *et al.* have suggested that single dose of 1000 µg of vitamin B12 is sufficient to meet increased demand of vitamin B12 in entire pregnancy (McLintock *et al.*, 2010; Clark *et al.*, 2007). The serum levels of vitamin B12 stabilize in four times the t-half time of vitamin B12, hence the response of the treatment was checked between day 25 - 30 (McLintock *et al.*, 2010). An increase in serum vitamin B12 levels was observed. The difference was found to be statistically significant (p value < 0.001). In pregnancy there is an increased requirement of substrates for haemoglobin synthesis like iron, folic acid and vitamin B12 consequent to increase in red cell mass and rapid cell growth in the growing fetus and placenta. In normal pregnancy by the third trimester, total serum vitamin B12 levels fall by 30% (Morkbak *et al.*, 2007; Myhre, 1964). Recommended intake of vitamin B12 during pregnancy is 2.6 µg daily (Pardo *et al.*, 2004). Unlike pernicious anaemia where vitamin B12 has to be supplemented lifelong, the increased demand of vitamin B12 during pregnancy lasts only for the duration of pregnancy and lactation. Vitamin B12 can be administered by various routes like oral, sublingual, intranasal and parenteral. Oral administration of vitamin B12 offers a potentially simpler and cheaper alternative to parenteral administration, but its effectiveness is unpredictable due to erratic absorption especially in malabsorption states (DeMayer and Tegman, 1998). Intramuscular injections of vitamin B12 seem to be a better modality of treating anemia due to vitamin B12 deficiency. Preparations designed for sublingual or intranasal administration are also available, but they are relatively expensive and there is not much research on their use. There are no robust studies on treatment of pregnant women with vitamin B12 deficiency anaemia as regards the dose, duration and mode of administration of vitamin B12. Hence there is no consensus regarding treatment of vitamin B12 deficiency anaemia in pregnant women.

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