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Original Article

Correlation of Serum Vitamin B 12 with Red Cell Indices and Hemoglobin in Indian Women in Second and Third Trimester of Pregnancy

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Abstract

Introduction: *To determine serum vitamin B 12 in pregnant women in India in second or third trimester and its correlation with red cell indices in order to determine an inexpensive screening tool to ascertain vitamin B 12 deficiency.*

Material & Methods: *In this prospective study, venous blood samples of consecutive 200 pregnant women in second or third trimester of pregnancy were included and their haematological and serum vitamin B 12 were determined including blood complete picture.*

Results: *Mean ± SD age of the subjects was 21.54 years (SD = 2.73). It was found that 72 had anemia. 25 (12.5%) women had vitamin B 12 levels below 647 pg/ml and were thus included. 37 had iron deficiency anaemia as their ferritin and iron levels were low and were thus excluded from the study. Significant correlation was seen between serum vitamin B 12 and Hb , PCV and MCHC. MCV and RDW did not show any significant correlation with vitamin B 12 levels.*

Conclusion: *We could not establish a statistical correlation between the all important MCV and vitamin B12 because it may be normal in combined deficiency of iron and vitamin B12/ folate or even in sublinical vitamin B12 deficiency. There is an urgent requirement to develop a non expensive marker to screen out patients of vitamin B12 deficiency as repeated vitamin B12 level estimation is expensive and cannot be affordable to majority in developing countries. Also, there is requirement to lay down reference values for vitamin B 12 during various phases of pregnancy.*

Keywords: *Vitamin B 12, Red Cell Indices, pregnancy.*

Introduction

Globally around 41.8% of the pregnant women are anaemic[1]. Nutritionally, iron deficiency is the main cause of anemia throughout the world during pregnancy. Folate and vitamin B12 deficiencies are the second most common cause of anemia after iron deficiency[2].

Centers for Disease Control and Prevention (CDC) has defined anemia in pregnancy as haemoglobin (Hb) level of less than 11 g/ dL during the I^st^ and III^rd^ trimesters and less than 10.5 g/ dL during the II^nd^ trimester[3]. The lower cut-off is due to haemodilution in pregnancy as plasma volume expands upto 30-40% as compared to 20-25% increase in red cell mass. This causes dilutional drop in Hb concentration creating a low viscosity state facilitating oxygen transport to the tissues including placenta. However, in India and most of the other developing countries the lower limit for Hb is often accepted as 10 gm per dL[4]. Also, there occurs physiological macrocytosis with average red cell size increase of 4 fL at term[5].

Vitamin B12 and folate due to its role in DNA synthesis and cell replication, it is considered essential for fetal growth and development[6]. Maternal vitamin B12 deficiency has been associated with increased risk of pregnancy complications, including spontaneous abortion, low birth weight, intrauterine growth restriction and neural tube defects[7]. Children born to vitamin B12-deficient women are at increased risk for developmental abnormalities and anaemia[8]. In a recent study in India, daily maternal vitamin B12 supplementation (50 µg/day) during pregnancy through 6 weeks postpartum substantially improved maternal vitamin B12 status and increased breast milk and infant plasma vitamin B12 concentrations[9].

The estimation of vit B 12 or folate is expensive and cannot be routinely done in all cases especially in developing countries. Therefore, surrogate markers need to be identified in women who require supplementation. It is also necessary to determine the utility of red cell indices in diagnosing their deficiency during pregnancy. This study was therefore done to ascertain utility of economical red cell indices to determine vitamin B 12 deficiency in pregnant women in India and to compare these to the established norms so as to determine whether the norms apply or whether there is a need to establish local and population specific norms.

Material and Methods

200 consecutive pregnant women in the second and third trimester (12-40 wks) were enlisted for this study. 25 non pregnant age matched healthy controls were also taken. Informed consent and Institutional ethical clearance was obtained.

Blood samples were collected EDTA (2 ml) and sterile vacutainer (3 ml). The EDTA anticoagulated sample was used for estimation of Hb and the red cell indices on Sysmex KX-21 three part semiautomated hematology cell counter. Hb, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), packed cell volume (PCV), red cell distribution width –standard deviation (RDW-SD) were tabulated. Wherever necessary, peripheral smear was seen to subtype the anemia. The serum was separated from the sample in sterile tube. Vitamin B 12 levels were estimated on automated chemiluminescence immune analyzer Roche (Hitachi) cobas e 411 using cobas kits. Value less than 182 pg/ml was considered as cut off. Simultaneous measurements of serum ferritin and iron were done and cases having concomitant deficiency were excluded.

Data was analyzed using Statistical Package for Social Sciences, version 15.0. Scatter plots were constructed between serum Vitamin B 12 and Hb, MCV, MCHC, PCV and RDW-SD%.

Results

The mean age of 200 consecutive pregnant women in second and third trimester of pregnancy was 21.54 years (SD = 2.73). As per CDC recommendation, a cut-off of Hb of 10.5 g/dL and 11 g/dL in 2nd and 3rd trimester respectively for anemia were considered and ferritin < 12 ng/mL were taken for presence of iron deficiency. It was found that 72 had anemia. 37 had iron deficiency anaemia as their ferritin and iron levels were low and were thus excluded from the study. 25 (12.5%) women had vitamin B 12 levels below

182 pg/ml and had normal serum ferritin and iron levels, were included.

The mean, median mode and SD of all Hb, red cell indices and serum vitamin B 12 of all cases were calculated. Significant correlation was seen between serum vitamin B 12 and Hb (Pearson's Corr coeff (r) = 0.272) (table 1) and PCV (Pearson's Corr coeff (r) = 0.271) (table 2). MCHC (Pearson's Corr coeff (r) = 0.198), p = 0.004) (table 3). MCV (Pearson's Corr coeff (r) = - 0.183) (table 4), and RDW (Pearson's Corr coeff (r) = -0.229) (table 5) did not show any significant correlation with vitamin B 12 levels.

We attempted to correlate between serum vitamin B 12 and red cell indices to see if any index can predict the presence of sub-clinical serum vitamin B 12 deficiency in this group but no significant correlation was found.

Table 1 Correlation between Vitamin B 12 and Hb

Vit B12	Haemoglobin		Total
	<11 gm/dl	≥ 11gm/dl	
< 182 pg/ml	2 (8)	23(42)	25
Total	2	23	25

Pearson's correlation coefficient = 0.272

Table 2 Correlation between Vitamin B 12 and PCV

Vit B12	PCV			Total
	<60fl	60-92fl	≥ 92fl	
< 182 pg/ml	25(100)	0	0	25(100)
Total	25(100)	0	0	25(100)

Pearson's correlation value = 0.271

Table 3 Correlation between Vitamin B 12 and MCHC

Vit B12	MCHC			Total
	< 20 gm/dl	20 – 33 gm/dl	≥ 33 gm/dl	
< 182 pg/ml	0	25(100)	0	25(100)
Total	0	25(100)	0	25(100)

Pearson's correlation value = 0.198

Table 4. Correlation between Vitamin B 12 and MCHC

Vit B12	MCV			Total
	<76 fl	76-96 fl	≥ 96 fl	
< 182 pg/ml	0	15(60)	10(40)	25(100)
Total	0	15	10	

Pearson's correlation value = - 0.183

Table 5 Correlation between Vitamin B 12 and MCHC

Vit B12	RDW		Total
	< 54 fl	≥ 54 fl	
< 182 pg/ml	17 (68)	8(32)	25
Total	17	8	25

Pearson's correlation value = - 0.229

Discussion

Anaemia in pregnancy is due to increased demand coupled with poor diet and many other reasons. A blood complete picture helps in evaluation and investigation of anaemia[10]. Mean Corpuscular Volume (MCV) in particular is of profound significance as it can help in identifying the cause of anaemia and is very cost effective. A low MCV is suggestive of iron deficiency anemia and raised MCV suggests vitamin B12 or folate deficiency. It is common practice that serum vitamin B12 and serum folate or red cell folate are estimated to confirm megaloblastic anemia. The levels of these are expected to be lower in such cases. Red cell folate is a more reliable maker than serum folate [11].

A normal MCV, however, is considered to imply that there is neither iron deficiency nor vitamin B12/ folate deficiency. On the contrary, apart from normal cases, MCV within its normal reference range can also occur in cases of combined mixed nutrient deficiencies[4,5]. Patients having deficiency of iron as well as vitamin B12/ folate exhibit an MCV lying within its normal range. Here RDW helps us in that if RDW is raised, it reflects anisopoikilocytosis and therefore smear examination is mandated[12,13]. Serum ferritin, vitamin B12 and red cell folate status can also be assessed and timely managed to avoid both maternal and foetal hazards[14, 15].

Cross-tabulation between the vitamin B12 and hemoglobin, PCV and MCHC was statistically significant in our study. However, negative correlation was seen in relation to MCV. This difference is attributed to the normal MCV values in patients with subclinical low ferritin, perhaps due to mixed micro nutrient deficiency[16]. As ferritin is also an acute phase reactant[17], it may have been falsely elevated, thus rendering a bias.

Studies conducted in general Indian population suggested up to 52% prevalence of vitamin B12 deficiency but only 2.4% of anemic patients to have increased MCV showing macrocytic picture, but surprisingly no studies report it during pregnancy. It is probably due to high prevalence of iron deficiency giving a picture of mixed deficiency anemia, thereby showing a normal MCV.[18]

Several studies suggested that vitamin B-12 status in women in resource-limited countries is poor. Among 113 Guatemalan women, plasma vitamin B-12 was deficient or low in nearly 50%, and breast milk vitamin B-12 concentration was low in 31%, [19]. In another study from Mumbai, mean plasma vitamin B-12 in pregnant non anemic women was 50.2 vs. 131.3 pmol/L in non pregnant women ($P < 0.001$)[9,20]. Of note, vitamin B-12 concentrations in all of the groups reported in these studies were generally low.

A study carried out in Venezuela, showed prevalence of folic acid and B12 deficiency in pregnant women of up to 36.32% and 61.34%. In this study the overall prevalence of severe anemia was 1.2%, out of which 41.6% of patients had macrocytic anemia[21].

It is poignant to note that, there are no different vitamin B 12 reference levels for pregnant women Several studies suggest that reference values for non pregnant women are not suitable for assessing vitamin B12 status during pregnancy, implying the need for reference values related to specific stages of pregnancy[12, 22].

Conclusion

MCV may be normal in combined deficiency of iron and vitamin B12/ folate or even in sublinical vitamin B12 deficiency. Therefore, it is essential that pregnant women with normal MCV should be screened for mixed deficiency anemia and further evaluation. There is an urgent need to develop a non expensive and readily accessible marker to screen out patients of vitamin B12 deficiency as repeated vitamin B12 level monitoring is expensive and cannot be affordable to majority in developing countries. Also, there is requirement to lay down reference values for vitamin B 12 during various phases of pregnancy.

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Conflict of Interests: All authors have none to declare

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