

The Role of Serum Vitamin B12 and Homocysteine in Recurrent Pregnancy Loss

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Abstract: The study was carried out from August 2012-2014 in Dr D.Y. Patil Hospital in the department of Obstetrics and Gynecology. 100 nonpregnant women with previous 2 or more abortions, attending OPD for BOH counseling were selected. They were subjected to serum B12, fasting serum homocysteine and folic acid measurements. Patients with B12 <200 pg/ml and homocysteine >15mmol/dl were identified. They were substituted with oral vitamin B12 till B12 levels were normalized. After this, the patients were asked to go ahead with conception and their subsequent obstetric course was studied. The prevalence of B12 deficiency was found to be 40%. After subsequent B12 substitution, 36 women conceived out of which 2 women had a repeated miscarriage at 8 weeks (5.5%). Out of these 36 women, 25 have had live births so far, while 6 have crossed 20 weeks uneventfully and 3 have crossed 12 weeks uneventfully at the time this study went into print. It was seen that B12 values were significantly lower and homocysteine values were significantly higher in study group.

Keywords: Recurrent pregnancy loss, Vitamin B12, Homocysteine, pregnancy outcome, dietary pattern

1. Introduction

The word abortion derives from Latin word "Aboriri" to miscarry. The National Centre for Health Statistics, Centre for Disease Control and Prevention and World Health Organization define abortion as pregnancy termination prior to 20 weeks gestation or with fetus born weighing less than 500 grams. Recurrent pregnancy loss has been inconsistently defined. Historically, it was defined as occurrence of 3 or more consecutive pregnancy losses. However, the American Society of Reproductive Medicine (ASRM) has recently redefined RPL as 2 or more pregnancy losses occurring consecutively. A clinically recognized pregnancy means, that pregnancy has been visualized on ultrasound or the chorionic tissue was identified after pregnancy loss. Overall, 12 – 15 % of clinically recognized pregnancies end in spontaneous miscarriage between 4 – 20 weeks of gestation¹. The risk of spontaneous pregnancy loss also depends on the woman's age. Risk is relatively low (7-15%) before age of 30. But rises sharply (34-52%) when maternal age reaches 40 or more. The prevalence of RPL is around 5% in couples attempting pregnancy. Recurrent pregnancy loss is classified as-

Primary RPL - When there is no live issue

Secondary RPL- When there is 1 or more live issue

The commonly implicated factors in RPL are : genetic factors (parental chromosomal anomalies), anatomical factors, endocrine abnormalities, infections, thrombophilia syndromes, autoimmune disorders, alloimmune disorders and exposure to environmental toxins.

In the past few years, vitamin B12 has been identified to play a pivotal role in recurrent pregnancy loss. The implicated mechanisms are^{2,3,4}-

1) B12 deficiency leads to faulty and sporadic ovulation producing a faulty oocyte.

- 2) B12 deficiency leads to a state of homocysteinemia which leads to thrombosis within the placental microcirculation.
- 3) B12 deficiency leads to incomplete trophoblastic invasion of spiral arteries thereby leading to defective placentation.

2. Methodology-

Sample size- 100 non pregnant women attending OPD for pre conceptional counselling

Study type- Prospective and interventional

Study duration- September 2012 to August 2014

Women with 2 or more recurrent pregnancy losses, who were not pregnant, and met the inclusion criteria were subjected to serum Vitamin B12 & fasting homocysteine levels estimation. Those women with low levels of Vitamin B12 and high level of homocysteine were identified. Women with consanguinous marriage, with BMI>25, with PCOS or other endocrine abnormality, with suspected malabsorption disorders and with an anomalous uterine cavity were excluded from the study.

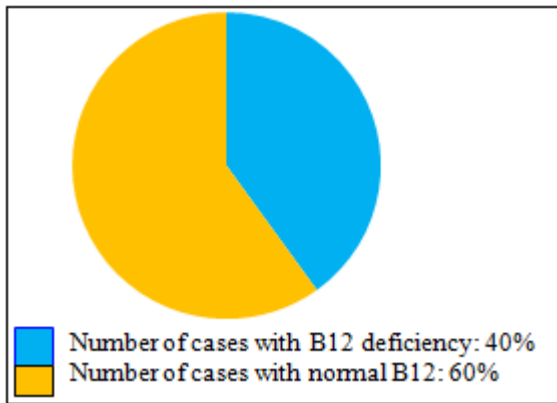
- Blood collection was done by venipuncture of antecubital vein. Sample was collected in plain blue.
- Serum B12 level, fasting serum homocysteine level and folic acid levels were measured. The method used was chemiluminescence assay.
- Those patients with Vitamin B12 levels less than 211 pg/ml were identified and labeled as B12 deficient (Normal Vitamin B12 level=211-800pg/ml). Also women who had serum homocysteine level homocysteine > 10 mmol/L were identified.(Normal level:5-15mmol/L)
- They were supplemented with oral Vitamin B12 tablets in the dose of 1500 micrograms for 2 months and serum B12 levels and fasting homocysteine levels were repeated.

- If serum B12 levels were found to be more than 300 pg/ml, they were allowed to try for pregnancy. If B12 levels were not yet replete, then B12 administration was continued till B12 and homocysteine levels were normalized.
- After serum B12 levels were brought back to above normal, that is, >300 pg/ml their subsequent obstetric course was studied.
- Their subsequent pregnancies were carefully followed up and classified into repeat abortion, full term gestation or preterm delivery. They were also vigilantly followed up for any pregnancy related complications like pregnancy induced hypertension or gestational diabetes.
- Simultaneously, 100 controls were also recruited. They were tested for Vitamin B12, Homocysteine and folic acid levels and their data was separately documented.
- Controls imply those women, with 1 or more healthy issue and no history of abortion.
- The mean serum B12, fasting homocysteine and folic acid levels in study and control population were compared and results were derived.

3. Results

Chi square test was applied for comparing percentages of different groups. All the calculations were performed by using the chi square test in Quikal software. Mean and standard deviation and its probability values were performed by using MS EXCEL software. Z test for proportion was applied using silico software, wherever applicable.

Out of 100 women with prior 2 or more pregnancy losses, taken up for study, 40 had low B12 levels. Thus, the incidence of B12 deficiency in our study was 40%.



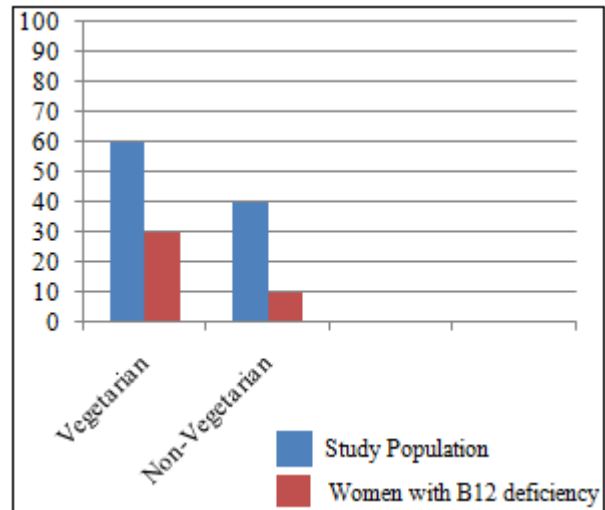
Graph 1

In our study, out of 100 women, 34 women (34%) were in between 20-25 years of age, majority i.e. 41 women (41%) were between 25-30 years of age while 25 women (25%) were between 30-35 years of age.

Out of 100 women with previous RPL 59 women had primary RPL meaning, they had no live issue while 41 women had Secondary RPL, meaning they had 1 or more live issue. 42 (42%) had 2 prior pregnancy losses, 35 women (35%) had 3 prior pregnancy losses, 14 women (14%) had 4 pregnancy losses while 9 women (9%) had more than 4

pregnancy losses prior to entering the study, the maximum number of RPL being 7.

61 women (61%) had miscarriages only in first trimester (upto 12 weeks), 19 women (19%) had miscarriage only in second trimester (12 – 20 weeks) while 20 women (20%) had miscarriages in both first and second trimesters. 60 had vegetarian dietary pattern while 40 had non-vegetarian dietary pattern. Out of 40 women, who were found to B12 deficient, 30 (75%) had vegetarian diet while 10 (25%) had non-vegetarian diet. This shows that B12 deficiency is commonly encountered in vegetarians. Out of 100 controls, 81 women had non-vegetarian diet, while 19 had vegetarian diet.



Graph 2

The 40 women with B12 deficiency were then given oral vitamin B12, in the dose of 1500 microgram/day till their serum vitamin B12 levels became normal. After their B12 levels were normalized they were permitted to conceive.

Out of 40, 36 women conceived naturally within 6 months. Out of these 36 pregnancies achieved, 2 ended in repeated abortion (5.5%), 25 (69.4%) ended in successful live birth, 6 (16.6%) have crossed 28 weeks uneventfully and 3 have crossed 20 weeks uneventfully (8.3%) at the time this study went into print.

Out of 25 live births, 2 (8%) had preterm delivery: one at 35 weeks due to PROM and other had preterm labor at 34 weeks due to Dengue fever. 5 women (20%) developed PIH after 30 weeks, 1 woman (4%) developed Gestational Diabetes Mellitus at 28 weeks and 1 woman (4%) had twin gestation., 17 women underwent full term vaginal delivery (68%) while 8 (32%) underwent Cessarean procedure.

Table 1: Fate of Pregnancy

Outcome of subsequent pregnancy	Number of women	Percentage
Repeat abortion	2	5.5%
Live birth	25	69.4%
Crossed 20 weeks	3	8.3%
Crossed 28 weeks	6	16.8%
Total	36	100%

Unpaired t test with Welch correction was applied for comparing mean B12, mean homocysteine and mean folic acid values in study and control group population. The mean B12 value in study population was significantly lower than mean B12 in control population. The mean homocysteine value in study population was significantly higher than mean B12 in control population. The mean folic acid value in study population did not differ significantly from the mean folic acid value in control population. This goes to prove that serum vitamin B12 deficiency was the attributable factor causing recurrent pregnancy loss in the study population.

4. Discussion

Vitamin B12 exists in a number of different chemical forms, all of which have a cobalt atom at the centre of a corrin ring. In nature, it exists as adenosine form (2 deoxyadenosyl) in the mitochondria. In the human plasma it exists in cell cytoplasm as methylcobalamin. It is a cofactor for methionine synthase. It is available in dairy products and non-vegetarian foods like meat. Daily requirements are 1-3 microgram/kg.

In the human body, B12 is a component of 2 important enzymes⁴-

1) Methionine Synthase

It plays an important role in 2 reactions

1. Conversion Of Homocysteine To Methionine-

This is the first step in the pathway by which 5 MTHF is converted into all the intracellular folate coenzymes.

2. Conversion Of 5-Methyl Tetrahydrofolates To Tetra Hydrofolates-

The enzyme folate polyglutamate synthase can only use Tetrahydrofolates and not 5 MTHF, as substrate. In cobalamin deficiency, MTHF accumulates in plasma while intracellular folate concentrations fall due to failure of formation of THF. This is called THF starvation or methylfolate trap. Hence, cobalamin deficiency can lead to abnormalities of folate metabolism as well leading to a state of pseudo folate deficiency.

2) Methylmalonyl Coenzyme (COA) Mutase

In Vitamin B12 deficiency, the activity of mutase is compromised, resulting in high plasma or urine concentrations of methylmalonic acid (MMA) which is a degradation product of methylmalonyl CoA. In adults, this mutase does not have any vital function, but in embryonic life and in early development, it has a very vital role.

Overall 12-15% of clinically recognizable pregnancies end in spontaneous miscarriage. The prevalence of RPL is between 15% in general population. Even after thorough evaluation, almost 20-25% of recurrent pregnancy losses remain unexplained. Vitamin B12 deficiency may be the underlying cause behind many such unexplained RPL.

In a similar study conducted by Votre P et al, 33% of women with prior pregnancy losses had low levels of

vitamin B12 and higher concentration of homocysteine. With subsequent administration of B12, their homocysteine concentration reduced, compared to those who received only folic acid or no supplementation⁵

In a study among South Indian Urban Women, 51.1% women showed impaired B12 serum concentration. Out of them, 64% were found to be strict vegetarians, with also low consumption of yogurt⁶.

Hubbner U et al conducted similar study in Syrian women with Recurrent Pregnancy loss. They confirmed that low levels of vitamin B12 were found in women with RPL. They found B12 deficiency prevalence around 38.4%⁷

5. Conclusion

Vitamin B12 is extremely important for healthy cell division and normal placental function. B12 deficiency leads to a state of homocysteinemia, causing thrombosis in placental microcirculation.

Due to pre-dominant vegetarian dietary pattern in our society and general neglect towards nutrition, the women in reproductive age group are prone to develop B12 deficiency which may be one of the important underlying cause of otherwise unexplained recurrent pregnancy loss. Serum B12 measurement is a relatively cheap and non-invasive procedure. The deficiency can be easily correctable by oral supplementation of vitamin B12. Hence, serum B12 measurement should be incorporated into the laboratory investigation profile of all the women with RPL.

6. Conflict of Interest

None declared

7. Acknowledgements

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References

- [1] Macklon NS, Geraedts JPM. Conception to ongoing pregnancy the black box of early pregnancy loss. *Journal of Human Reproduction* 2002; 8:333-343
- [2] Willianne L.D, Johan Bulten, Eric Steegers *Journal of Human Reproduction* 15 (4) 954-960, 200
- [3] Katve P et al: vitamin B12 and folic acid supplementation and plasma total homocysteine concentration in pregnant Indian women with low B12 status. *Asia Pacific Journal of Clinical Nutrition* 2010, 19 (3) 335-43.
- [4] Lap Pez, Vaisca MA *European Journal of Obstetrics and Gynecological Reproductive Biology* 2003 May 1, 108 (1) : 45-9.
- [5] A Victor Hoffbrand Megaloblastic anemic Vit B12 absorption. *Harrison's principles of Internal Medicine* Vol. I, 17th Edition 2008 : 645 Votre et al Hyperhomocysteinemia and early abortion and

- pregnancy complications. ClinChem Lab Med 2005; 43 (10) : 1052 – 7
- [6] Katve P et al Vitamin B12 and folic acid supplementation and total plasma homocysteine in pregnant women with low B12 status. Asia Pacific Journal of Clinical Nutrition 2010 19 (3) : 335-43.
- [7] Hubbner U, Samual TM, Duggan C, Thomast, Bosch T, et al Ann. NutrMetab 2013 62 (2) : 113 – 22.