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**Original Research Article**

# Role of serum folic acid and vitamin B12 levels in abruptio placentae and the fetomaternal outcome

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## ABSTRACT

**Background:** Over the course of pregnancy, there is a steady decline in maternal plasma folate concentration and vitamin B12 concentration about 50%of non pregnant levels. Insufficient folate and vitamin B12 status has been associated with many reproductive complications including abruption, IUGR, pre eclampsia, early pregnancy loss. The aim of the study was to determine the role of serum folic acid and vitamin B12levels in patients with abruptio placentae and to study the feto-maternal outcome in these patients. Feto maternal outcome is seen in terms of mode of delivery whether vaginal or caesarean, period of gestation whether term or preterm, need of blood transfusion, stillbirths.
**Methods:** In this prospective observational study conducted in a tertiary hospital from 2018-2020, 50 pregnant women with abruptio placentae were included and their serum folic acid and vitamin B12 levels were measured by ELIZA method using commercially available kits.
**Results:** Serum folic acid levels were not low in the cases of placental abruption with range= 25 ng/ml to 80.5 ng/ml. Mean folic acid level ±SD is 47.98±13.15 ng/ml and median is 48 ng/ml. In this study vitamin B12 levels were low in the cases, range 14 pg/ml to 70 pg/ml. Mean vitamin B12 value ±SD is 27.15±11.63 pg/ml and median is 25 pg/ml.The rate of caesarean section was 44%, preterm delivery was 64% and stillbirth was 38% in these cases with mean folic acid levels of 48.7±15.4 ng/ml, 46.94±13.85 ng/ml and 46.03±8.13 ng/ml respectively and mean vitamin B12 levels of 23.34±6.74 pg/ml, 28.73±13.44 pg/ml and 28.32±11.75 pg/ml respectively. There was a significant association (p=0.006) between vitamin B12 and mode of delivery. No other significant association was seen between serum folic acid and vitamin B12 levels and the different fetomaternal outcome.
**Conclusions:** Low levels of vitamin B12 is seen in cases with abruptio placentae. The rate of caesarean section, preterm delivery, stillbirth and need of blood transfusion is high but no significant association is seen.

**Keywords:** Folic acid, Vitamin B12, Placentae, Fetomaternal

## INTRODUCTION

The human placenta is a window for both health and disease of the mother and her developing embryo. Placental abruption is defined as premature separation of the placenta from the decidua at or after 20 weeks of gestation. Placental abruption refers to separation of placenta partially or totally from its implantation site before delivery. It can be either revealed with bleeding insinuating between membranes and uterus ultimate lyescaping through the cervix to cause external haemorrhage or it can be concealed with blood.[1] Placental abruption has been associated with an increase in the risk of stillbirth, preterm delivery, haemorrhage, need for

hysterectomy, DIC and death. Placental abruption is a clinical diagnosis, typically characterized by vaginal bleeding, abdominal pain, uterine contractions and/or tenderness, and sometimes non- reassuring foetal monitoring.[2] Short interpregnancy intervals may also contribute to depletion of maternal folate status, leading to adverse outcomes in later pregnancies.[3] Inadequate maternal folate status has been linked to abruptio placentae, preeclampsia, spontaneous abortion, stillbirth, preterm delivery, and low birthweight. Perhaps the strongest associations have been with abruptio placentae. The total plasma homocysteine concentration, which is a sensitive marker of folate status, has been implicated as a risk factor for abruptio placentae and increased risk of abruptio placentae has been linked to polymorphisms in folate related genes, suggesting that even a mild dysfunction in folate metabolism might predispose to these events.[4] Vitamin B12 and folic acid act as co enzyme in the metabolism of homocysteine which is derived from demethylation of methionine. It is a metabolite in methionine-cysteine pathway. It is metabolized in body to either cysteine using pyridoxine (vitamin B6) or it can be recycled to methionine using folic acid and methyl cobalamin (Vitamin B12) as co factors.[5] In a normally functioning metabolic state, methionine produces homocysteine as an intermediate step before either transsulfuration via cystathionine into cysteine or remethylation to methionine. This remethylation may be folate-dependent, or may use betaine, a metabolite of choline. Methionine synthase, a vitamin $B_{12}$-dependent enzyme, utilizes 5-methyltetrahydrofolate as the carbon donor for folate-dependent homocysteine remethylation; methionine synthase requires activation by methionine synthase reductase (MTRR). Betaine-homocysteine methyltransferase (BHMT) utilizes betaine as the carbon donor. Therefore, the improper function of remethylation enzymes, due to mutation or to insufficient intake of relevant nutrients, may result in elevated homocysteine levels and contribute to thrombophillias, and possibly, placental abruption.[6,7] High circulating total homocysteinemia itself increase the risk of a wide range of abnormalities in vascular function, most likely through increased oxidative stress leading to endothelial cell dysfunction. In pregnancy this is seen primarilyas affecting placental function. Elevated total homocysteine has been associated with a variety of adverse outcomes linked to placental insufficiency, including preeclampsia, spontaneous abortion, abruptioplacentae, intrauterine growth restriction, recurrent pregnancy loss and preterm birth.[8] The reaction with N-5 MTHF occurs in all tissues and is vitamin B12 dependant, whereas the reaction with betaine is confined mainly to the liver and is vitamin B12 independent. Folic acid and vitamin B12 supplementation may further reduce the total homocysteine concentration during pregnancy. Dietary deficiency of these micronutrients, mutation in MTHFR gene or cystathionine-beta synthase is associated with increase in serum homocysteine levels, this explains the close relation between folic acid, vitamin B12 and homocysteine.[9] Folic acid and vitamin B12 along with other nutritional, genetic, physiological and pathological causes are involved in the elevated serum homocysteine levels. Placental development in early pregnancy may be negatively influenced by increased maternal homocysteine concentrations. Moderately elevated homocysteine concentrations may induce cytotoxic and oxidative stress, leading to endothelial cell impairment.[5] Additionally, exposure of trophoblast cells to homocysteine may increase cellular apoptosis and lead to inhibition of trophoblastic function. Homocysteine is thought to be related to early placentation, so it may affect subsequent foetal growth. Placental vasculopathy might be associated with preterm birth.[1,10] In India, studies on folic acid and vitamin B12, in relation to abruption are very few. Decreased level of folic acid and vitamin B12 might be an independent factor associated with abruption. It could be possible to bring health benefits to patients by treating deficiency of folic acid and Vitamin B12, if it is proved to be associated with abruption and therefore improve the feto maternal outcome. The goal of the present study was to examine whether maternal plasma folic acid and vitamin B12levels, measured in third trimester of pregnancy, are associated with abruption and their effect on maternal and fetal outcome.

### *Objectives*

Objectives of current study were to determine the serum levels of folic acid and vitamin B12 in patients with abruptio placentae and to study feto-maternal outcome in these patients.

## METHODS

The prospective observational study was conducted in the department of obstetrics and gynaecology of a tertiary care hospital in collaboration with department of biochemistry for a period of 18 months. Informed and written consent was obtained from all participants in a language which the understood. All the information procured from the study was kept confidential and used for academic purposes only.

### *Inclusion criteria*

All women diagnosed as cases of abruption placentae both clinical and on USG will be included in the study. Women with gestational age >28 weeks, all women in whom there is antepartum confirmed sonographic diagnosis of placental abruption>28 weeks before or during delivery and all delivered women whose placenta showed retroplacental haematoma/clots were included in the study.

### *Exclusion criteria*

Women with diagnosed placenta previa, premature rupture of membranes, multiple pregnancy, history of thromboembolism, uterine leiomyomas with a diameter >5 cm, genital tract malignancy, medical co-morbid condition DM, hypertension, polyhydramnios, history of repeated

miscarriage, history of anaemia, history of preterm labour, history of smoking, prior significant history, vitamin B12 and folic acid supplementation and taking antifolate drugs or steroids were excluded.

### *Sample size*

The study of Bouzari et al observed that mean serum homocysteine in case was 10.76±1.55. Takingthis value as reference, the minimum required sample size with estimate to be within 0.5 and 5% level ofsignificance is 37 patients. To reduce margin of error, total sample size taken is 50. Formula used is:

$$N \geq ((SD * Z_{\alpha})/ME)^2$$

Where $Z_{\alpha}$ is value of Z at two sided alpha error of 5%, ME is margin of error and SD is standard deviation, thus by applying above formulae sample size was calculated to be 37.

### *Procedure*

Eligible pregnant women were recruited from labour room and wards. Informed consent was taken, thorough clinical examination was done and history was taken, prior investigations like ultrasound were studied. Following investigations were performed in all patients complete blood count, KFT, LFT, coagulation profile, urine albumin, serum TSH, serum levels of homocysteine using commercially available kits.

Blood sample collection: blood samples were collected with all aseptic precaution from antecubital vein of the subjects. Approximately 5ml blood was collected. Samples were centrifuged to separate cells and plasma. Folic acid determination: folic acid determination was done by ELISA method using commercially available kit. The kit uses a double-antibody sandwich enzyme-linked immunosorbent one-step processassay (ELISA) to assay the level of folic acid in samples. Vitamin B12 determination: vitamin B12 determination was done by ELISA method using commercially available kit. The kit uses a double-antibody sandwich enzyme-linked immunosorbent one-step process assay (ELISA) to assay the level of vitamin 12 in samples. Investigations: blood group, Rh typing, complete blood count, urine pregnancy test, liver function test, renal function test, coagulation profile, blood sugar, thyroid function test, obstetric ultrasonography (if available) with dates and details and serum folic acid and vitamin B12 levels.

### *Outcome measures*

Primary outcome of the study: Proportion of women with abruptio placentae having increased levels of homocysteine. Secondary outcome of the study: It was assessed as; maternal outcome in terms of; Number of vaginal delivery, Caesarean section, Haemodynamic instability/ shock, No of blood transfusion, number of FFP transfused, number of women who went into DIC and Maternal death. Foetal outcome in terms of: Prematurity and IUD, FGR and NICU admissions.

### *Statistical analysis*

Categorical variables were presented in number and percentage (%) and continuous variable were presented as mean ±SD and median. Normality of data was be tested by Kolmogorov- Smirnov test. If the normality is rejected then non parametric test was used. Statistical tests were applied as follows: Quantitative variables were compared using Unpaired t-test/Mann-Whitney test (when the data sets were not normally distributed) between the groups and qualitative variables were compared using Chi-Square test/Fisher's exact test. The data was entered in MS excel spreadsheet and analysis was done using statistical package for social sciences (SPSS) version 21.0.

## RESULTS

The observational study was conducted in the department of obstetrics and gynaecology at a tertiary care hospital on 50 patients who had abruption and they were recruited after taking informed consent. The study was conducted over a span of 18 months. The observations were recorded in the master chart and were evaluated using Statistical Package for Social Sciences (SPSS) version. On studying the serum folic acid and vitamin B12 levels of 50 patients with placental abruption following observations were made: Serum folic acid levels were found to be elevated in all cases. Range =25 ng/ml to 80.5 ng/ml. Mean folic acid level ±SD is 47.98±13.15ng/ml and median is 48 ng/ml. Serum vitamin B12 levels were low in the cases and the minimum and maximum vitamin B12 levels are 14 pg/ml and 70 pg/ml respectively. Mean vitamin B12 value ±SD is 27.15+-11.63 pg/ml and median is 25 pg/ml. The rate of caesarean section was high with 44% cases with mean serum folic acid and vitamin B12 level of 48.7±15.4ng/ml and 23.34±6.74 pg/ml respectively in these cases. There was no significant association (p=0.734) between serum folic acid levels and mode of delivery. There was a significant association (p=0.006) between vitamin B12 and mode of delivery. The rate of stillbirths was high with 38% cases with mean serum folic acid and vitamin B12 level of 46.03±8.13 ng/ml and 28.32±11.75pg/ml in these cases. There was no significant association (p=0.351, p=0.406) between serum folic acid and vitamin B12 levels and stillbirths. The rate of preterm delivery was high with 64% cases with mean serum folic acid and vitamin B12level of 46.94±13.85 ng/ml and 28.73±13.44pg/ml respectively in these cases. There was no significant association (p=0.839, p=0.485) between serum folic acid and vitamin B12 level and preterm delivery. In our study, blood transfusion was given in 96% cases, retroplacental clots were present in 60% cases. No other adverse feto maternal outcome was found to be associated with elevated serum folic acid and vitamin B12 levels.

## DISCUSSION

Insufficient folate status has been associated with many reproductive complications including anovulation, sub-fertility, and early pregnancy loss. Homocysteine is commonly used as an indicator of folate status, an amino acid involved in one-carbon metabolism of folate. Over the course of pregnancy, there is a steady decline in maternal folate concentration to 50% of nonpregnant levels along with the increased placental and foetal demands.[2] In this study, study the folic acid levels were not low in the cases and the minimum and maximum folic acid levels are 25 ng/ml and 80.5 ng/ml respectively. Mean folic acid ±SD is 47.98±13.15 ng/ml and median is 48 ng/ml. Folic acid levels were found to be more than the standard normal range, reason being would be the practise of supplementing folic acid in preconception and early pregnancy period in every ANC patient as per the existing hospital protocol. Serum vitamin B12 levels were low in the cases and the minimum and maximum vitamin B12 levels are 14 pg/ml and 70 pg/ml respectively. Mean vitamin B12 value ±SD is 27.15±11.63 pg/ml and median is 25 pg/ml. The serum folic acid and vitamin B12 levels decreased significantly ($p<0.001$) through pregnancy and reached lowest value in third trimester. 11.1% of gestations resulted in IUGR, 7.9% in GDM, 4.8% in GHTN and remaining patients had complications like abruption, IUD, etc.[11] In a cohort of 1228 women with 1-2 previous pregnancy losses and no documented infertility, a block randomized, double blind placebo controlled trial was done whereby women were randomized to daily low dose aspirin (81 mg/dl) or placebo and all women received folic acid (400 µg/day). No meaningful relationships were found between serum folate and any reproductive outcome (anovulation, pregnancy loss).[12] Caesarean delivery is often necessary in the setting of placental abruption to limit further oxygen deprivation to the foetus, to reduce blood loss for the mother and, in extreme cases, to prevent the death of the foetus, the mother, or both, Spontaneous preterm birth due to abruption is thought to be the result of bleeding from the separation of the placental which irritates the uterine lining and stimulates contractions which progress into preterm labour.[13] The association between abruption and foetal growth is likely to be a reflection of underlying chronic placental ischemic disease, which reduces the oxygen and nutrient availability to the foetus, thereby stunting foetal growth. It is also possible that a milder, partial separation of the placental could occur earlier in gestation, thereby directly reducing supply to the foetus, while not triggering a preterm birth. It is plausible that perinatal deaths that are not due to preterm birth are attributable to asphyxia in the setting of abruption.[14]

In this study, no significant association was found between feto maternal outcome (such as mode of delivery, preterm delivery, low birth weight, stillbirth, need of blood transfusion) with serum levels of folic acid and vitamin B12 levels. The rate of preterm delivery was high with 64% cases with mean serum folic acid level of 46.94±13.85 ng/ml and mean serum vitamin B12 level of 28.73±13.44 pg/ml in these cases. Blood transfusion was given to 96% cases, retroplacental clots were present in 60% cases. In a study by KL Downes et al, a systemic review of 123 studies were reviewed and abruption was associated with elevated risk of caesarean delivery, postpartum haemorrhage and transfusion, preterm birth, intrauterine growth restriction or low birth weight, perinatal mortality, and cerebral palsy. Caesarean delivery risk ranged from 2.4 to 61.8 (incidence range: 33.3-91%) and was the most frequently reported delivery outcome associated with abruption. Estimated risk for preterm delivery ranged from 1.2 to 31.7 with incidence ranging from 5.8 to 80.1%. Stillbirth was seen frequently with abruption with risk ranging from 3.4-51.8%. IUGR/SGA was reported to occur in 2-40% of abruption cases with estimated risk ranging between 1.3 to17.4. In a retrospective cohort study by Boisrame et al 247 cases of abruption and controls were included. The rate of caesarean section was 90.3% and of foetal death was 78%.[15] In a study by Faiz, cox proportional hazards model was used to estimate the risk of stillbirth associated with maternal risk factors and pregnancy complications. It was seen that adjusted relative risks for the risk factors associated with stillbirth were 40.2 (95% CI 36.9-43.9) for placental abruption, 5.3 (95% CI 3.4-8.2) for eclampsia, 1.7 (95% CI 1.3-2.2) for preeclampsia and 3.5 (95% CI 2.8-4.3) for diabetes mellitus.[16] In a multivariate logistic regression analysis by Tikkanen et al 198 women with abruption and 396 controls were included. 59% had preterm labour (OR 12.9, 95% CI 8.3-19.8), 91% were delivered by caesarean section (OR 34.7, 95% CI 20-60.1), perinatal mortality rate was 9.2% (OR 10.1, 95% CI 3.4-30.1) and retroplacental clots were seen by ultrasound in 15% of Cases.[17] In a retrospective cohort study by GS Macheku, 39,993 women were included and it was seen that feto maternal complications associated with abruption were APH (OR 11.5, 95% CI 6.3-21.2), PPH (OR 17.9, 95% CI 8.8-36.4), caesarean delivery (OR 5.6, 95% CI 3.6-8.8), need for blood transfusion (OR 9.6, 95% CI 6.5-14.1), LBW (OR 5.9, 95% CI 3.9-8.7), perinatal death (OR 17.6, 95% CI 11.3-27.3).[18] In a cross sectional study by Gul et al 334 patients with APH were included. Proportion of abruption among these patients was 20.6%, out of which 7.5% underwent caesarean section. Association between placental abruption and caesarean section was found significant with $p=0.03$.[19] Therefore it was concluded that risk of caesarean section is increased in pregnancies complicated with abruption.

## CONCLUSION

Serum folic acid levels were not low in the cases of placental abruption with the minimum and maximum serum folic acid levels of 25 ng/ml and 80.5ng/ml respectively. Mean folic acid level ±SD is 47.98±13.15ng/ml and median is 48 ng/ml.In this study vitamin B12 levels were low in the cases and the minimum and maximum vitamin B12 levels are 14 pg/ml and 70 pg/ml respectively. Mean vitamin B12 value ±SD is

27.15±11.63 pg/ml and median is 25 pg/ml. The rate of caesarean section was 44%, preterm delivery was 64% and stillbirth was 38% in these cases with mean folic acid levels of48.7±15.4 ng/ml, 46.94±13.85 ng/ml and 46.03±8.13 ng/ml respectively and mean vitamin B12 levels of23.34 6.74 pg/ml, 28.73±13.44 pg/ml and28.32±11.75 pg/ml respectively. Retroplacental clots were present in 60% of cases and blood transfusion was received by 96%. There was a significant association (p=0.006) between vitamin B12 and mode of delivery. No other significant association was seen between serum folic acid and vitamin B12 levels and the different fetomaternal outcome.

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## REFERENCES

1. Cunningham F, Leveno K, Bloom S, Gilstrap L, Cunningham F. Williams Obstetrics. New York, USA: McGraw- Hill Professional Publishing; 2010;25:767-70.
2. Per MU, Stein EV. Homocysteine and Folate in Pregnancy. Clin Chem. 2004;50;1293-95.
3. Nilsen RM, Vollset SE, Rasmussen SA, Ueland PM, Daltveit AK. Folic acid and multivitamin supplement use and risk of placental abruption: a population-based registry study. Am J Epidemiol. 2008;167(7):867-74.
4. Mukhopadhyay I, Pruthviraj V, Biswas M. Hyperhomocysteinemia in recurrent pregnancy loss and the effect of folic acid and vitamin B12 on homocysteine levels: a prospective analysis. Int J Reprod Contracept Obstet Gynecol. 2017;6:2258-61.
5. Avinash K, Palfrey HA, Pathak R, Kadowitz PJ, Gettys TW, Murthy SN. The metabolism and significance of homocysteine in nutrition and health. Nutr Metab. 2017;14:23-9.
6. Katre P, Bhat D, Lubree H, Otiv S, Joshi S, Joglekar C, Rush E. Vitamin B12 and folic acid supplementation and plasma total homocysteine concentrations in pregnant Indian women with low B12 and high folate status. Asia Pac J Clin Nutr. 2010;19:335-43.
7. Ananth CV, Elsasser D, Kinzler WL, Peltier MR. Polymorphisms in Methionine Synthase Reductase and Betaine-Homocysteine S-Methyltransferase Genes: Risk of Placental Abruption. Mol Genet Metab. 2007; 91:104-10.
8. Jaiswal N, Agrawal A, Agrawal S, Das V, Singh R. Correlation of serum homocysteine levels and pregnancy outcome: the dilemma continues. Int J Reprod Contracept Obstet Gynecol. 2016;5:391-95
9. Ray JG, Laskin CA. Folic acid and homocysteine metabolic defects and the risk of placental abruption, pre-eclampsia and spontaneous pregnancy loss: A systemic review. Placenta. 1999;20:519-29.
10. Micle O, Muresan M, Antal L, Bodog F, Bodog A. The influence of homocysteine and oxidative stress on pregnancy outcome. J Med Life. 2012;5:68-73.
11. Ubeda N, Reyes L, Medina AG, Aperte EA, Moreiras GV. Physiologic changes in homocysteine metabolism in pregnancy: A longitudinal study in Spain. Nutrition. 2011;27:925-30.
12. De Vilbiss EA, Mumford SL, Sjaarda LA, Connell MT, Kim K, Mills JL, et al. Preconception folate status and reproductive outcomes among a prospective cohort of folate-replete women. Am J Obstet Gynecol. 2019; 221:151-10.
13. Youssry M, Radwan A, Gebreel M, Patel T. The impact of third trimester maternal serum vitamin B12 and folate status on fetal birth weight. is maternal serum homocysteine a predictor of low birth weight infants?. J Obstet Gynecol. 2017;7:1102-15.
14. Jiang H, Cao L, Chen H. Blood folic acid, vitamin B12, and homocysteine levels in pregnant women with fetal growth restriction. Genet Mol Res. 2016;15:1-8.
15. Boisrame T, Sananes N, Fritz G, Boudier E, Aissi G, Favre R et al. Placental abruption: risk factors, management and maternal-fetal prognosis. Cohort study over 10 years. Eur J Obstet Gynecol Reprod Biol. 2014;179:100-4.
16. Faiz AS, Demissie K, Rich DQ, Kruse L, Rhoads GG. Trends and risk factors of stillbirth in New Jersey 1997-2005. J Matern Fetal Neonatal Med. 2012;25: 699-705.
17. Tikkanem M, Nuutila M, Hiilesmaa V, Paavonen J, Ylikorkala O. Clinical presentation and risk factors of placental abruption. Acta Obstet Gynecol Scand. 2006; 85:700-5.
18. Macheku GS, Philemon RN, Oneko O, Mlay PS, Masenga G, Obure J, et al. Frequency, risk factors and feto-maternal outcomes of abruptio placentae in Northern Tanzania: a registry- based retrospective cohort study. BMC Preg Childbirth. 2015;15:242.
19. Gul S, Abrar S, Jamal T, E Rana Gul, Majid A, Iqbal M. Association between placental abruption and caesarean section among patients at Khyber teaching hospital Peshawar. J Ayub Med Coll Abbottabad. 2016;28:172-4.

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