INTERNATIONAL JOURNAL OF CURRENT ADVANCED RESEARCH
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 12; Issue 05(A); May 2023; Page No. 2063-2067
DOI: http://dx.doi.org/10.24327/ijcar.2023.2067.1452

Research Article

ROLE OF SERUM HOMOCYSTEINE LEVELS IN ABRUPTIO PLACENTAE AND THE FETOMATERNAL OUTCOME

Seema Meena, Richa Choudhary, Pragati Meena and Neha Vashisth*

VMMC and Safdarjung Hospital New Delhi

A R T I C L E I N F O

Article History:
Received 06th February, 2023
Received in revised form 14th March, 2023
Accepted 23rd April, 2023
Published online 28th May, 2023

Key words:

High serum homocysteine

A B S T R A C T

Background and objective: High serum homocysteine level is considered as a risk factor for placental abruption. Placental development in early pregnancy may be negatively influenced by increased maternal homocysteine concentrations. Moderately elevated homocysteine concentrations may inducecytotoxic and oxidative stress, leading to endothelial cell. impairment. Additionally, exposure of trophoblast cells to homocysteine may increase cellular apoptosis and lead to inhibition of trophoblastic function. Maternal plasma homocysteine concentration at various stages during normal pregnancy tends to be lower than in nonpregnant women. Placentalabruption has been associated with an increase in the risk of stillbirth, preterm delivery, haemorrhage, need for hysterectomy, DIC and death. The aim of the study was to determine the role of serum homocysteine levels in patients with abruptio placentae and to study the fetomaternal outcome in these patients. Fetomaternal outcome is seen terms of mode of delivery whether vaginal of caesarean, period of gestation whether term or preterm, need of blood transfusion, stillbirths, early neonatal deaths and NICU admission.

Methods: In this observational study, 50 pregnant women with abruptio placentae were included. 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 studiedand their serum homocysteine levels were measured by ELIZA method using commercially available kits. The obtained data was statistically analysed using Statistical Package for Social Science (SPSS) version 21.0.

Results: Serum homocysteine levels were found to be elevated in all cases, ranging from 32.0 µmol/L to 165 µmol/L and mean homocysteine level +-Std. deviation is 62.57 +-21.79 µmol/L. The rate of caesarean section was 44%, preterm delivery was 64% and stillbirth was 38% in these cases with mean homocysteine levels of 65.1± 28.08µmol/L, 61.02 ± 18.12 µmol/L and 62.84 ± 23.89µmol/L respectively. Retroplacental clots were present in 60% of cases and blood transfusion was received by 96%. No significant association was seen between serum homocysteine levels and the different fetomaternal outcome.

Conclusion: Hyperhomocysteinemia 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.

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 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 ultimately escaping through the cervix to cause external haemorrhage or it can be concealed with blood. 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[1].

Homocysteine is a naturally occurring amino acid, derived from demethylation of methionine requiring folate, vitamin B12, B6 as a co enzyme. 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[2].

Corresponding author:* **Seema Meena
VMMC and Safdarjung Hospital New Delhi

Metabolism of homocysteine is at the interaction of two metabolic pathways: re methylation and trans sulfration. In re methylation homocysteine acquires a methyl group from N-5 Methyl tetrahydrofolate or from betaine to form methionine. Methionine synthase reductase (MTRR) and Betaine homocysteine S-methyltransferase (BHMT) are 2 enzymes that regulate homocysteine metabolism[2,3]. 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. Maternal plasma homocysteine concentration at various stages during normal pregnancy tends to be lower than in non pregnant women. These changes have not been completely explained by hemodilution or alterations in renal function or albumin concentration and are probably also mediated by endocrine changes and changes in vitamin status. Folic acid supplementation may further reduce the total homocysteine concentration during pregnancy.

Dietry 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[3].

Hyperhomocysteinemia is classified as[4]:

- Moderate (homocysteine level 15-30 micromol/L)
- Intermediate (homocysteine levels 30-100 micromol/L)
- Severe (homocysteine level >100 micromol/L)

Pathophysiology

Placental development in early pregnancy may be negatively influenced by increased maternalhomocysteineconcentrations. Moderately elevated homocysteine concentrations may inducecytotoxic and oxidative stress, leading to endothelial cell impairment. 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.]

Elevated plasma total homocysteine can arise not only from inadequate folate or vitamin B12status, but also from a range of other nutritional, genetic, physiological and pathological causes. 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 primarily as affecting placental function[6].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].

In India, studies on homocysteine, in relation to abruption are very few. Increased level of homocysteine might be an independent factor associated with abruption. It could be possible to bring health benefits to patients by treating hyperhomocysteinemia, 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 homocysteine levels, measured in third trimester of pregnancy, are associated with abruption and their effect on maternal and fetal outcome.

Aims and Objectives

To determine the association of hyperhomocysteinemia with abruptio placentae.

Estimation of serum levels of homocysteine in patients withabruptio placentae and to study feto-maternal outcome in these patients

MATERIAL AND METHODS

The present study was conducted in the Department of Obstetrics and Gynaecology, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, in collaboration with Department of Biochemistry. STUDY DESIGN - Observational study, STUDY PERIOD-18 months STUDY SETTING-Department of Obstetrics and Gynaecology, VMMC and SJH, New Delhi STUDY POPULATION- All women diagnosed as cases of abruption placentae both clinical and on USG will be included in the study.

Inclusion Criteria

1. Women with gestational age >28 weeks
2. All women in whom there is antepartum confirmed sonographic diagnosis of placental abruption>28 weeks before or during delivery.
3. All delivered women whose placenta showed retroplacental haematoma/clots.

Exclusion Criteria

Womens with

- diagnosed placenta previa • premature rupture of membranes • Multiple pregnancy
- history of thromboembolism • uterine leiomyomas with a diameter >5cm
- 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 • taking antifolate drugs or steroids

Consent-informed and written consent was obtained from all participants in a language which the understood.

Sample Size

The study of Z Bouzari, *et al* observed that mean serum homocysteine in case was 10.76
± 1.55. Taking this value as reference, the minimum required sample size with estimate to be within .5 and 5% level of significance 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_{α} is value of Z at two sided alpha error of 5%, ME is margin of error and SD is standard deviation. Calculations:- $n>=((1.55*1.96)/.5)^2=36.92=37$(approx.)

METHODOLOGY

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[7].

Homocysteine Determination: homocysteine 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 homocysteine in samples. The kit has enzyme wells which are pre coated with homocysteine antibody to which standard, test sample and HRP labelled homocysteine antibody are added followed by incubation for 60 minutes at 37 degree C. Then plate is washed for 5 times and chromogen A and B solution is added followed by incubation for 15 minutes at 37 degree C. On adding chromogen solution A and B, the colour of the liquid changes into blue and the reaction with the acid causes the colour to become yellow. Normal range of serum homocysteine is 3.2-21.4 micromol/L.

History

- Demographic profile of women including age, religion, residence, (rural/urban), education, per capita monthly income, Menstrual history-LMP, EDD according to LMP, frequency, duration, quantity, associated with dysmenorrhea or not. History of antenatal supervision; if yes, gestational age at first and last check-up. If no, reasons.
- Obstetric history including gravity, parity, number of living children, age of last living birth, mode of delivery of each child, if underwent LSCS then indication for the same, any complication during previous pregnancy including abruption, birth weight of previous children, are they alive and healthy, previous stillbirths, birth defects or pregnancy losses, repeated miscarriage, preterm labour. Any history of consanguinity of marriage
- Past history of any febrile illness with or without rash, torch infection, hypertension, DM, liver or renal disease, heart disease, thyroid disease.
- Drug history including intake of folic acid or vitamin supplementation antenatally, anti-folate drug, steroids, anticoagulants. Family history of any medical disorder like hypertension, DM, abruption, coagulation defect. Personal history- Any history of passive or active smoking and any history of substance abuse. Confirmation of gestational age by first or second trimester USG or by last menstrual period.
- Antenatal complications including preeclampsia or eclampsia, gestational hypertension, Gestational diabetes mellitus (GDM), Intrahepatic cholestasis of pregnancy (IHCP), APH, FGR. If yes, whether diagnosed antenatally or not.
- Gestational age at delivery in weeks. Diagnosis at admission, singleton or multiple pregnancy, whether foetal heart sound. FHR was present at admission or not, labour details and any malpresentation. Mode of delivery and any antepartum, intrapartum or postpartum complications (obstructed labour, rupture uterus and cord complications).
- Foetal characters including gestational age (in weeks), sex, weight (in grams), and any visible birth defects. In case of stillbirth, whether fresh or macerated.

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., Serum homocysteine levels.

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

- No.of vaginal delivery • Caesarean section • Haemodynamic instability/ shock
- No of blood transfusion• No. of FFP transfused
- No. of women who went into DIC• Maternal death

Foetal outcome in terms of

- Prematurity and IUD• FGR• NICU admissions

Ethical Issues

- Informed Consent - A written and informed consent was taken from all the enrolled women in a language well understood by them (ANNEXURE 3)
- Institutional Ethics Committee (IEC) Approval - Approval from IEC was obtained before starting the study.
- Confidentiality - All the information procured from the study was kept confidential and used for academic purposes only.

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.
- 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.

Table 1 Comparison of results of various studies

S.NO	Author(Year) Number	Type of Study	Remarks
1	Z Bouzari *et al* (2014)[20] Cases 60 Control 60	Case control	Abruption was found to be associated with increased serum homocysteine levels and showed that the role of hyperhomocysteinemia as a risk factor for abruption.
2	S H Choudhary *et al* (2019)[21] N=7587	Retrospective cohort study	An increase inhomocysteine concentrationsignificantly increased the odds of any placenta-mediated complication OR for a 5 µmol/L increase: 1.63, 95% CI 1.23–2.16).
3	Jaiswal *et al* (2016)[8] Cases=39 Control=42	Prospective study	Serum homocysteine levels were higher in the women with pregnancy complication like abruption, preeclampsia and abortion as compared to women without complication but the difference was not statistically significant. (p=0.403)
4	Cande V Ananth *et al* (2007)[9] Cases-196 Control -191	case control study	DNA was genotyped for the MTRR and BHMT polymorphisms. The rate of homozygous mutant BHMT genotype was 2.8-fold (OR 2.82, 95% CI 1.84, 4.97) higher in cases than controls.
5	Hordaland homocysteine study (2000)[8] 53 cases 47control	Case control study	87.5% of cases with hyperhomocysteinemia had adverse outcome like IUGR, spontaneous abortion and preterm birth.
6	In this study (2020) 50 cases	Prospective observational study	Serum homocysteine levels were found to be elevated in all cases. Range = 32.0 µmol/L to 165 µmol/L. Mean homocysteine level +-Std. deviation is 62.57 +- 21 µmol/L. and median is 61.25 µmol/L.

DISCUSSION

Elevated maternal plasma homocysteine level is proposed to be associated with placentamediated pregnancy complications. In this study serum homocysteine levels were found to be elevated in all cases with the minimum and maximum homocysteine levels- 32.0µmol/L and165µmol/L respectively. Mean homocysteine level +-Std. deviation is 62.57+- 21.79µmol/L and median is 61.25µmol/L. In a cohort study by S H Choudhary *et al* 7587 cohort participants were included in the study. Maternal plasma homocysteine concentration was significantly associated (linearly) with an increased risk of both the composite outcome of any placenta-mediated complication (including placental abruption, preeclampsia, foetal growth restriction and pregnancy loss) ($p = 0.0007$), SGA ($p = 0.0010$), severe SGA, and marginally with severe preeclampsia, but not preeclampsia, placental abruption and pregnancy loss. An increase in homocysteine concentration significantly increased the odds of any placenta-mediated complication (odds ratio (OR) for a 5 µmol/L increase: 1.63, 95% Confidence Interval(CI) 1.23–2.16) and SGA (OR 1.76, 95% CI 1.25–2.46)[2,7]. In a case control study by CandeV. Ananth *et al* Women with a clinical diagnosis of abruption were recruited as incident cases (n=196), and controls (n=191) were matched to cases based on maternal race/ethnicity and parity. Total plasma homocysteine concentrations were evaluated in a subset of 136 cases and 136 controls. DNA was genotyped for the MTRR and BHMT polymorphisms. The rate of homozygous mutant BHMT genotype was 2.8-fold (OR 2.82, 95% CI 1.84, 4.97) higher in cases than controls. In a case control study by Z Bouzari *et al* a significant difference (p<0.001) was observed between the serum homocysteine levels in cases and control and showed the role of hyperhomocysteinemia as a risk factor for abruption[5,6]. In a prospective study by Jaiswal *et al* conducted in Lucknow, 81 patients were taken and they were followed till delivery. Out of these, 42 women had an uncomplicated pregnancy and delivery and remaining 39 women had at least one antenatal or perinatal complication like abortion, preeclampsia, placental abruption.

Difference between mean serum homocysteine in both the groups was not statistically significant (p=0.403). Though the serum homocysteine levels were higher in the women with pregnancy complication as compared to women without complication but the difference was not statistically significant.[9,10]

RESULTS

The observational study was conducted in the department of Obstetrics and Gynaecology at Safdarjung 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 homocysteine levels of 50 patients with placental abruption following observations were made:

- Serum homocysteine levels were found to be elevated in all cases. Range = 32.0 µmol/L to 165 µmol/L. Mean homocysteine level +-Std. deviation is 62.57 +- 21.79 µmol/L and median is 61.25 µmol/L.
- The rate of caesarean section was high with 44% cases with mean serum homocysteine level of 65.1 ± 28.08 µmol/L in these cases. There was no significant association (p= 0.845) between serum homocysteine levels and mode of delivery.
- The rate of stillbirths was high with 38% cases with mean serum homocysteine level of 62.13 ± 18.46µmol/L in these cases. There was no significant association (p=0.92) between serum homocysteine levels and stillbirths.
- The rate of preterm delivery was high with 64% cases with mean serum homocysteine level of 61.02 ± 18.12µmol/L, mean in these cases. There was no significant association (p=0.839) between serum homocysteine level and preterm delivery.
- In our study, blood transfusion was given in 96% cases, retroplacental clots were present in 60% cases.

- No adverse feto maternal outcome was found to be associated with elevated serum homocysteine levels.

CONCLUSIONS

Serum homocysteine levels were elevated in the cases of placental abruption with the minimum and maximum homocysteine levels of 32.0µmol/L and 165µmol/L respectively. Mean homocysteine level +-Std. deviation is 62.57 +- 21.79 µmol/L and median is 61.25µmol/L .The rate of caesarean section was 44%, preterm delivery was 64% and stillbirth was 38% in these cases with mean homocysteine levels of 65.1± 28.08µmol/L, 61.02 ± 18.12µmol/L and 62.84 ± 23.89µmol/L respectively. Retroplacental clots were present in 60% of cases and blood transfusion was received by 96%. No significant association was seen between serum homocysteine levels and the different fetomaternal outcome.

References

1. Micle O, M Muresan, L Antal, F Bodog, A Bodog. The influence of homocysteine and oxidative stress on pregnancy outcome. J Med Life 2012; 5:68-73.
2. Ubeda N, Reyes L, Medina A G, Aperte E A, Moreiras G V. Physiologic changes in homocysteine metabolism in pregnancy: A longitudinal study in Spain. Nutrition 2011; 27:925-930.
3. De Vilbiss E A, Mumford S L, Sjaarda L A, Connell M T, Kim K, Mills J L *et al*. Preconception folate status and reproductive outcomes among a prospective cohort of folate-replete women. Am J Obstet Gynecol 2019; 221:151-10.
4. 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?. Open Journal of Obstetrics and Gynecology 2017; 7:1102-15.
5. 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.
6. 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 ObstetGynecolReprodBiol 2014; 179:100-4.
7. Faiz AS, Demissie K, Rich DQ, Kruse L, Rhoads GG. Trends and risk factors of stillbirth in New Jersey 1997-2005. J MaternFetal Neonatal Med 2012; 25:699-705.
8. Tikkanem M, Nuutila M, Hiilesmaa V, Paavonen J, Ylikorkala O. Clinical presentation and risk factors of placental abruption. Acta ObstetGynecolScand 2006; 85:700-705.
9. 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 Pregnancy Childbirth 2015;15:242.
10. 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.

How to cite this article:

Seema Meena, Richa Choudhary, Pragati Meena and Neha Vashisth (2023) 'Role of Serum Homocysteine Levels in Abruptio Placentae and the Fetomaternal Outcome', *International Journal of Current Advanced Research,* 12(05), pp. 2063-2067.
DOI: http://dx.doi.org/10.24327/ijcar.2023.2067.1452

****