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STUDY OF MATERNAL SERUM HOMOCYSTEINE LEVELS AS A PREDICTOR OF PLACENTA MEDIATED COMPLICATIONS

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Abstract:

Background: One of the major causes of adverse Fetomaternal outcomes have been found to be the various Placenta mediated pregnancy Complications (PMCs). There are inconsistent studies that confirms or proves elevated Serum Homocysteine concentration to be one of the risk factors for Placenta Mediated Complications. Prospective and sufficiently powered studies that uses the pathophysiology of the disease as the basis of their study will help and encourage for a better management of women and fetuses at risk. Hence, this study focuses to determine whether elevated maternal plasma Homocysteine levels in early to mid-second trimester has any significant difference on the occurrence of Placenta-Mediated Complications (PMCs).

Objectives: To study and compare the occurrence of Placenta mediated Complications and their Fetomaternal Outcome in women having normal and elevated Serum Homocysteine levels.

Methodology: Females in their early second trimester (Gestational age within 12 -20 weeks) with singleton pregnancy irrespective of taking or not taking periconceptional folic

acid, presenting for prenatal care in the ANC clinic or planning to deliver at AVBRH Hospital will be selected. Fasting Blood samples will be taken in EDTA Vacutainer tubes and transported to the laboratory within 30min and then the maternal Serum Homocysteine levels will be estimated using the Enzyme-Linked Immunosorbent Assay. Women with raise Plasma Homocysteine Levels will be categorized into Group A and women with Normal Plasma Homocysteine Levels will be categorized into Group B. Pregnancy outcomes and complications will be extracted by studying the antenatal, perinatal, and postnatal medical records and comparison will be made between both the groups for the level of significance.

Results: The Expected Outcome of this study will be a significant difference in the occurrence of Placenta Mediated Complications and related Adverse Fetomaternal Outcomes in women having elevated and normal Serum Homocysteine Levels.

Conclusion: Our Study will prove the role of maternal homocysteine levels in occurrence of Placenta-mediated pregnancy Complications.

Keywords: Placenta-Mediated Complications (PMCs), Homocysteine, Fetomaternal outcomes

Introduction:

In developing countries like India around 15% of women develop Placenta Mediated Complications. Out of all these, Placenta Mediated Complications (PMCs) account for around 5-15%, resulting in significant maternal and perinatal adverse outcomes. Early Diagnosis and Treatment of these complications is essential, as reducing maternal and infant mortality has been enshrined to be one of the Millennium Development Goals (MDGs).(1)

Impaired placental function during Pregnancy has various manifestations, primarily FGR (Fetal Growth Restriction) or Small for Gestational age (SGA), Hypertensive disease like Preeclampsia, abruptio placentae, Early and Recurrent loss of pregnancy.(2)

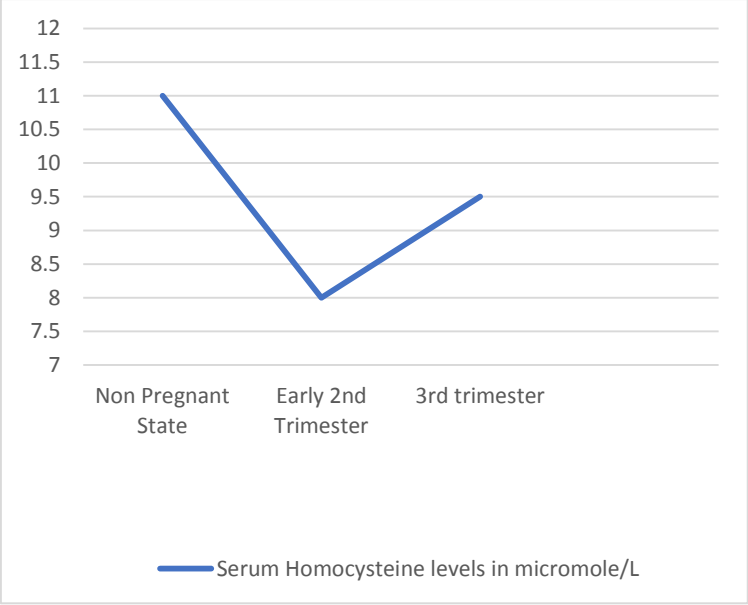
Various markers like APLA (Anti Phospholipid Antibody), LMWH (Low Molecular Weight Heparin), Serum Vitamin B12 levels, Serum Folic acid levels have been studied in the past to establish an association with Placenta mediated pregnancy complications (PMCs), but results remain controversial and inconsistent. According to Recent studies, Serum Homocysteine concentration is being found to be associated with various placenta mediated pregnancy complications but there is still inconclusive results on the degree to which maternal hyperhomocysteinemia is a causative factor of Placenta Mediated Complications.(3)

Homocysteine is an amino acid formed from methionine which is a sulfur containing amino acid involved in methylation and sulfuration pathways(4). It is an intermediate metabolite implicated in damaging the endolelial layer of blood vessels and thereby is a cardiovascular risk factor. During the course of normal human pregnancy, homocysteine levels fall. The etiology behind this decrease in homocysteine concentration during pregnancy includes , the normal increase in plasma volume and the haemodilution , the normal increase in the glomerular filtration rate (GFR), and a possible increase in the uptake of homocysteine by the fetus.(5)

The involvement of Homocysteine in the pathology of various Diseases in Humans is explained by hypothesis, that includes Homocysteinylation causing cytotoxicity, oxidative stress and neurotoxicity.(6) The mechanism by which Homocysteine plays a role in developing Obstetrical Complications is found to be associated to early placentation which involves early invasion and vascularization of trophoblast. Increased maternal homocysteine concentrations have shown to affect Placental development in early pregnancy. Alteration in maternal homocysteine levels causes placental vasculopathy which leads to the various Placenta mediated Complications(7).

It is essential and of great importance to maintain metabolism of homocysteine and to keep Serum Homocysteine concentration at an appropriate level. It has been found on research that raised levels of serum Homocysteine is not only limked to pathophysiology of both vasculopathy and cardiovascular diseases but also a risk factor of some severe pregnancy related complications which threaten the lives of both the mother and the new born. It has been reported that preeclampsia patients have higher levels of serum HCY than healthy pregnant women found in early second trimester of pregnancy. Serum Homocysteine levels reflect how severe the various Placenta Mediated Complications could be. Furthermore, researchers have also studied the impact of elevated Maternal Plasma Homocysteine levels on normal labor and baby's birth. High levels of Homocysteine results in placental dysfunction, leading to early and recurrent pregnancy loss, abruptio placentae and intrauterine growth restriction.

In the second trimester of pregnancy Serum Homocysteine levels are the lowest and it increases in late third trimester of pregnancy. Therefore, samples must be taken in the early second trimester. Sample collection during this time frame would give a better success at correlating the homocysteine levels with various Fetomaternal outcomes. Also as we know most of the maternal complications such as preeclampsia and gestational diabetes develop much later in pregnancy so if the samples are collected in the above given strict time frame it would sufficiently precede the onset of various Placenta mediated Pregnancy Complications (8).



There are inconsistent study results reported that prove association between maternal homocysteine concentration, measured during each of the three trimesters of pregnancy and

placenta-mediated complications; even among larger studies. With above background, in present study we focus on evaluation of the role of raised Serum Maternal Homocysteine concentrations in predicting the occurrence of various Placenta Mediated Complications.

Aim: We aim to study and compare the occurrence of Placenta mediated Complications and their Fetomaternal Outcome in women having normal and elevated Serum Homocysteine levels estimated in early second trimester so as to analyse the effect of elevated Maternal Homocysteine levels on the development of adverse pregnancy outcomes and to find out the role of Serum Homocystiene level screening in prediction and prevention of Placenta Mediated Complications and their poor Fetomaternal Outcomes.

Objectives:

1. To study the occurrence of Placenta Mediated Complications In women with normal Homocysteine Levels estimated in early second trimester.
2. To study the occurrence of Placenta Mediated Complications in women with Raised Homocysteine Levels estimated in early second trimester.
3. To compare between above two groups.

Material And Methods:

Study Design- Prospective Observational Study

Duration of Study- 2020-2022

Sample Size- Estimated Sample size is **900** based on study duration,
Calculated by using the following formula

$$\boldsymbol{n = \frac{4pq}{L^2}}$$

where

p = proportion of PMC (Placenta mediated Complications) = 10
q = 100 - p
L = Allowable Error = 20% of p = 2

Inclusion Criteria:

- Antenatal Women giving consent to participate in the study
- All Antenatal Women in early second trimester (12 to 20wks Gestational Age)
- Women with Singleton Pregnancy

Exclusion Criteria:

- Women not giving consent for study
- <12wks Gestational Age
- >20weeks Gestational Age
- Twin or Multiple Pregnancy
- Chronic Hypertension

Place of Study-Department of Obstetrics and Gynaecology, Acharya Vinobha Bhave Rural Hospital (AVBRH), Datta Meghe Institute of Medical Sciences (DMIMS), Sawangi (Meghe), Wardha.

Ethics approval-Ethical clearance from the Institutional Ethical Committee will be taken before enrolling patient into the study.

Enrollment-

- Women fulfilling the inclusion criteria will be selected as participants.

- That includes Women in their early second trimester (Gestational age within 12 - 20 weeks) with singleton pregnancy irrespective of taking or not taking periconceptional folic acid, presenting for prenatal care in the ANC clinic or planning to deliver at AVBRH Hospital.

- Every woman will be explained the type and the nature of the study and informed written consent will be taken.

- Age, Parity, geographical origin, maternal comorbidities like hypertension, thyroid disease, diabetes, heart disease, history of previous pregnancies including abortions, Obstetric history, Menstrual history along with relevant medical history will be recorded. History of Maternal habits, like consumption of alcohol, caffeine and smoking will be taken.

- Physical and obstetric examinations, laboratory findings will be noted. Investigations like Complete Blood Count, Blood Grouping, Liver Function Test, Kidney Function Test, Urine Examination, Blood Sugar, Thyroid Function test will be done. Along with routine antenatal investigations, Pregnancy Induce Hypertension(PIH) investigations like BT, CT, APTT, PT, INR, fibrinogen, urine albumin, Serum Lactate Dehydrgenase(LDH) and serum uric acid will also be done.

- Obstetric Ultrasonography will be done to see for fetal viability, gestational age, effective Fetal weight, biparietal diameter, head circumference, abdominal circumference, femur length, placental position, amniotic Fluid Index and a Doppler examination done on uterine artery, umbilical artery and medial cerebral artery.

Intervention-

- Fasting Blood samples will be taken in EDTA Vacutainer tubes and transported to the laboratory within 30min and then the maternal Serum Homocysteine levels will be estimated using the Enzyme-Linked Immunosorbent Assay.
- Serum Homocysteine levels are done at Acharya Vinoba Bhave Rural Hospital, Sawangi (Meghe), Wardha and to cover the cost we shall be applying for funding from Intramural grant/ ICMR grant/ Concession for Synopsis.

Categorization-

- Women with raise Plasma Homocysteine Levels will be categorized into G**roup A** and women with Normal Plasma Homocysteine Levels will be categorized into **Group B**.

- Both the Groups will be followed Antenatally, Intrapartum and Postpartum to see for development of various Placenta Mediated Complications.

Diagnosis & Comparison-

- **FIGO (International Federation of Gynaecology & Obstetrics)** has described Placenta Mediated Complications as Great Obstetric Syndromes/Placental Syndromes that includes (9)
  -Hypertensive disorders in pregnancy (HDP)- preclampsia
  -Fetal growth restriction (FGR)/Small for Gestational Age (SGA)
  -Intrauterine fetal death (IUD)
  -Stillbirth
  -Preterm birth
  -early and recurrent pregnancy loss.
- Details of number of participants developing Placenta Mediated Complications will be obtained by studying the antenatal, perinatal, and postnatal medical records and the outcomes will be noted and comparison will be made between both the groups for the level of significance.

Outcomes-

Primary outcomes

Maternal-

- Preeclampsia
- Abruptio Placentae
- Early Pregnancy Loss
- Recurrent Pregnancy Loss

Fetal-

- Fetal Growth Restriction (FGR) or SGA (Small for Gestational Age)
- Intrauterine Fetal Death (IUFD)
- Preterm births
- Still Births
- Neural tube Defects (NTDs)

Secondary Outcomes

Maternal-

- Oligohydramnios
- Meconium Stained Amniotic Fluid
- Ante partum Hemorrhage
- Postpartum Hemorrhage
- Mode of delivery
- Maternal Mortality
- Unexplained Infertility

Fetal-

- NICU Admission
- Low APGAR Score
- Requirement of resuscitation

Expected Results:

The Expected Outcome of this study will be the significant difference in the occurrence of Placenta Mediated Complications and related Adverse Fetomaternal Outcomes in women having elevated and normal Serum Homocysteine Levels.

Discussion:

Mortality of the mother and the newborn is the most severe adverse outcome of pregnancy. Complications or problems during pregnancy, at delivery or during the postpartum period can result in adverse maternal or infant outcomes. Increased maternal homocysteine concentrations in early pregnancy have been found to affect placental development. The link between Hyperhomocysteinemia and various obstetrical complications is very recent and there are conflicting results in proving elevated maternal serum homocysteine levels as the new obstetrical risk factor.(10)

It was found In Ottawa and Kingston (OaK) Birth cohort, that maternal serum homocysteine concentration in early to mid-second trimester was associated with increased chances of development of any of the placenta-mediated complications (PMC).(11)

Bergen et al.'s cohort also found that increased early second trimester homocysteine was linked with an increase in the risk of Placenta mediated pregnancy complications. Whereas in Dodds et al.'s cohort, a high maternal homocysteine concentration was not related to increased incidence of Placenta mediated Complications. Also nested case-control study by Kahn et al., found no association between homocysteine levels and Placenta Mediated Complications.

According to the study of Ottawa and Kingston cohort, there was not much difference in the development of placenta mediated complications in women taking or not taking folate supplementation. However, according to some studies there has been an increased risk of Placenta Mediated Complications in women with higher homocysteine concentrations not on periconceptional folic acid. Many of these studies were done when routine supplementation of peri-conceptional folic acid was not mandatory or were conducted in countries where folic acid fortification is not done.(12) This indicates that when intake of folic acid is low, homocysteine levels will be high and therefore homocysteine might play a prime role in the occurrence of Placenta Mediated Complications.

Study by Wadhwani et al. came to conclusion that high levels of serum homocysteine from 16th week of pregnancy is associated with increased risk of development of Preeclampsia later in the course of pregnancy suggesting that measuring the concentration of serum Homocysteine early in pregnancy before the occurrence of pregnancy related complications will be of benefit in early diagnosis and management of mother and fetus at risk. More detailed and larger studies are required to better illustrate the role of raised Homocysteine levels in the pathology of Preeclampsia and other placenta mediated complications. Also this study suggested that folate and vitamin B12 supplementation during pregnancy was of benefit for normal and healthy Fetomaternal outcomes.

As Placenta mediated Complications still remains a major cause of poor fetomaternal outcomes and often result in death of mother and new born (13). A number of studies reflect on similar issues (14-17). Few articles related to this study were reviewed(18-20). If the proposed study sufficiently demonstrates a good correlation between raised Serum Homocysteine levels and Placenta Mediated Complications, it could be included in routine

investigations of antenatal care and screening for Placenta Mediated Complications and also aid in early detection, management and vigilance in patient care.

Conclusion:

Our Study will suggest a significant difference in the development of Placenta Mediated Complications in women with raised serum Homocysteine Levels in early to mid-second trimester and thereby will prove the role of maternal homocysteine levels in occurrence of placenta-mediated pregnancy complications.

Treatment options to reduce the levels of Homocysteine are safe and inexpensive. Hence it is important to continue studies which establish strong association between hyperhomocysteinemia and placenta mediated complications so that we will be able to determine the mechanism by which elevated homocysteine levels causes obstetrical complications and we can get potential benefits of the treatment.

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