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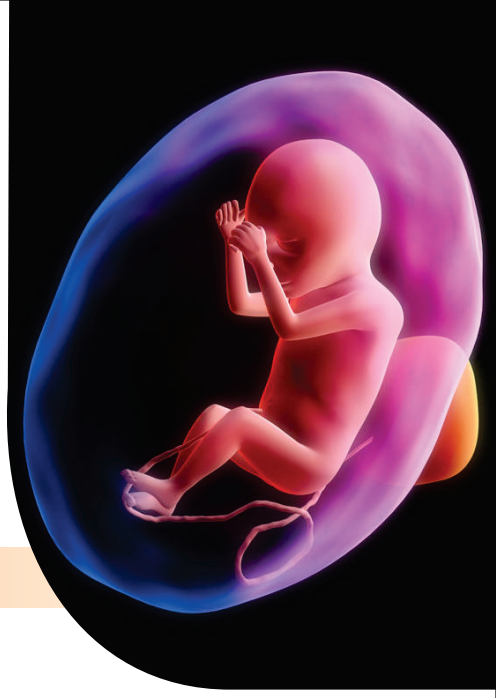


THROMBOPHILIA:

ANOTHER FACTOR TO CONSIDER IN CASES OF RECURRENT PREGNANCY LOSS

Department of Molecular Biology

INTRODUCTION:

Recurrent pregnancy loss exacts a devastating emotional toll on patients' lives. Each miscarriage brings with it a profound sense of loss and frustration. While hormonal, uterine, immune system, and chromosomal abnormalities are widely accepted as possible causes of repeat miscarriages, the latest studies point to a new area of investigation - inherited blood clotting factors.

When a patient has a tendency to form blood clots, the condition is called thrombophilia. Thrombophilia can be a life-threatening event if the clots restrict blood flow. Thrombophilia can be an inherited disorder, but can also be caused by external events such as surgery, obesity, pregnancy, use of oral contraceptives, antiphospholipid syndrome, or long periods of immobility. Physicians may suspect thrombophilia when patients have a blocked blood vessel at a young age or have a strong family history of clotting disorders

Recent research suggests a possible correlation between inherited thrombophilia and recurrent fetal loss. **Genetic markers for these clotting factors include factor V Leiden mutation, MTHFR mutation (677 & 1298) prothrombin G20210A mutation.** These three mutations are the most common causes of inherited thrombophilia. These markers, as well as several others that have also been **associated with recurrent miscarriage,** can be detected through simple blood tests

In patients with Thrombophilia, **damage of the placenta happens mostly because of blood clot formation into the maternal as well as the fetal vessels.** In addition, because the maternal blood comes out of the maternal vessels and flows very slowly between the branches of the fetal vessels (intervillous space), maternal blood is vulnerable to clot formation. If maternal blood clots in this space, then the fetal vessels will be destroyed because there will not be enough nutrients and oxygen for them. That leads to placental degeneration and fetal vessel damage. **Impaired placental development has been associated with the following conditions:**

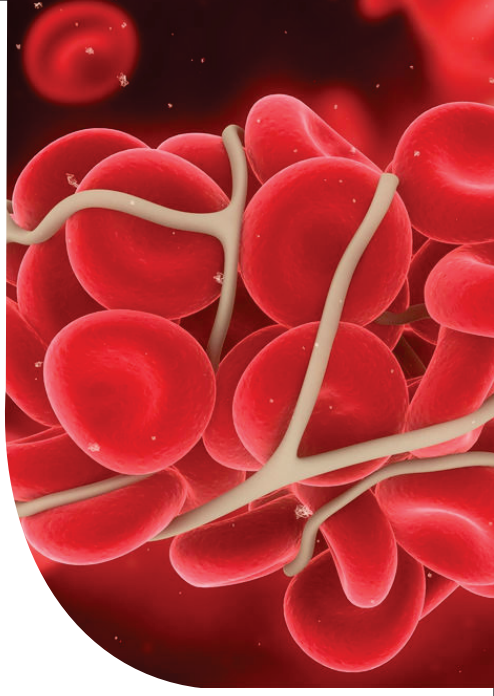
- Failure of the embryo to attach to the uterine lining (implantation).
- Miscarriages
- Growth failure of the fetus because of insufficient placental growth.
- Hypertensive disorders of the pregnancy (Preeclampsia/Toxemia).
- Decreased amniotic fluid volume (Oligohydramnios).

- Partial or complete separation of the placenta (Abruptio placentae).
- Silent premature cervical changes that may lead to premature delivery.
- Pre-term labor and delivery.
- "Unexplained"intrauterine fetal demise (death).
- Severe fetal deprivation of oxygen, which may lead to variable degrees of mental problems including cerebral palsy.
- Clots may form within the maternal veins and that can lead to thrombophlebitis or deep vein thrombosis, which can be dangerous for the mother.

GENETIC THROMBOPHILIA MARKERS AND MISCARRIAGE

Factor V Leiden – is a protein **involved in the normal blood clotting process.** Once the blood has sufficiently clotted, the Factor V Leiden protein becomes inactivated. Some people inherit a defective Factor V Leiden gene (a point mutation). This genetic defect makes the Factor V Leiden protein much more difficult to inactivate, resulting in excessive clotting. **The Factor V Leiden defect has been associated with: recurrent pregnancy loss, late abortions, fetal death/growth retardation (IUGR)** and pregnancy complications such as pre-eclampsia (a condition in pregnancy characterized by a sharp increase in blood pressure). One study found that **19% of miscarriage** patients (15 of 80) **carried the factor V Leiden mutation**.

MTHFR – is a recessive gene that leads to an accumulation of **high levels of homocysteine that subsequently promotes**



thrombophilia. Typically, **homozygous MTHFR** gene needs to have the mutation in order to have a significant impact. Mutations of the **MTHFR gene are associated with a significant risk for recurrent pregnancy loss (RPL) and birth defects.** It has also been linked to third trimester and post-partum complications.

Prothrombin – a protein in the blood that is required in order for it to clot. A mutation in the Prothrombin gene results in excess production of the Prothrombin protein, making the blood more likely than normal to clot. Pregnancy complications from this mutation include: recurrent pregnancy loss (RPL), **placental abruption** (a condition in which the placenta detaches to some extent from the wall of the uterus), **fetal death and pre-eclampsia**
(a condition in pregnancy characterized by a sharp increase in blood pressure).

Plasminogen Activator Inhibitor – 1 (PAI-1) – is an enzyme involved in the process of breaking down blood clots. Elevated levels of this enzyme are associated with excessive clotting. **Abnormal levels of PAI-1 are associated** with pregnancy complications such as: **recurrent pregnancy loss (RPL), pre-eclampsia** (a condition in pregnancy characterized by a sharp increase in blood pressure), fetal growth retardation and fetal death.

INDICATIONS OF TESTING:

- Evaluation of all patients with venous thrombosis, coronary artery disease, and/or stroke of unknown etiology.
- Evaluation of asymptomatic individuals with a family history of venous thrombosis.
- Evaluation of individuals with family members known to have Factor V Leiden, Prothombin G2010A, MTHFR C677T or MTHFR A1298C mutations.
- **Evaluation of women with recurrent pregnancy loss, unexplained severe pre-eclampsia, placental abruption, fetal growth retardation, still birth or neural tube defects in offspring.**

METHEDOLOGY OF TESTING:

Allelic Discrimination by TaqMan Assay (Applied Biosystems) is used to determine the genotype at each of the above loci. End-products are analyzed using the ABI 7500 Real-Time PCR System for genotype detection.

SENSITIVITY OF ASSAY:

This test methodology detects >99% of instances of these mutations.

SPECIMEN REQUIREMENT:

At least 2ml whole blood in lavender top (EDTA) tube. Label tube with patient's name, age and date of collection. Phlebotomist must initial tube to verify patient's identity.

TAT AND ORDERING INFORMATION:

TEST NAME	METHOD	SPECIMEN	REPORTING TIME
FACTOR V LEIDEN MUTATION DETECTION	REAL TIME PCR	EDTA WB	48 Hours
MTHFR GENE MUTATION DETECTION	REAL TIME PCR	EDTA WB	48 Hours
PROTHOMBIN GENE MUTATION	REAL TIME PCR	EDTA WB	48 Hours
THROMBOPHILLIA PCR PANEL*	REAL TIME PCR	EDTA WB	48 Hours
PAI-1 GENOTYPING	PCR SEQUENCING	EDTA WB	7 Days

* Thrombophillia PCR panel includes Factor V Leiden G1691A Mutation, MTHFR C677T & A1298C Mutations and Prothombin G20210A Mutation.

RESULTS:

Each test report includes a detailed interpretation of the genetic findings, the clinical significance of the result, and specific recommendations for clinical management and additional testing, if warranted. Results will be reported to the referring physician or health care provider as specified on the test requisition form.

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