

An interesting case report of Association of Recurrent Pregnancy Loss with MTHFR gene mutation and high Anti phospholipid antibodies

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Abstract: Recurrent pregnancy loss (RPL) is defined as the loss of three or more first consecutive pregnancies before 20 weeks' gestation. In most of the cases underlying cause remains unknown. We report an interesting case of a woman with recurrent pregnancy loss associated with heterozygous mutation in the MTHFR gene, high levels of antiphospholipid antibodies and significantly low vitamin D levels.

Keywords: Recurrent Pregnancy Loss, Antiphospholipid antibodies, MTHFR gene mutation

INTRODUCTION

Recurrent pregnancy loss (RPL) is defined as the loss of three or more consecutive pregnancies before 20 weeks' gestation [1]. Epidemiologic studies have revealed that 1% to 2% of women experience RPL [2]. In nearly 50% of patients with RPL, the underlying cause remains unknown.

Most of the spontaneous miscarriages are caused by an abnormal karyotype of the embryo. At least 50% of all first trimester spontaneous abortions are cytogenetically abnormal with many gene mutations like MTHFR. One of the most important treatable cause for RPL may be presence of antiphospholipid antibodies (APLAs), which have reportedly been positive in 10-20% of women with early pregnancy loss. Three classes of clinically significant APL antibodies have been identified: anticardiolipin (ACL), lupus anticoagulant (LAC), and anti- β_2 glycoprotein I antibodies [3]. Many recurrent miscarriages are characterized by defective placentation and microthrombi in the placental vasculature. Here we present a case of woman with recurrent pregnancy loss associated with MTHFR gene mutation, high antiphospholipid antibodies and significantly low vitamin D levels.

CASE REPORT

A 36 year old woman presented to the OP of our institute with a previous history of three first trimester pregnancy loss (at 8, 11 and 12 weeks of gestation) without any treatment. Physical examination was normal and there was no similar history in the family.

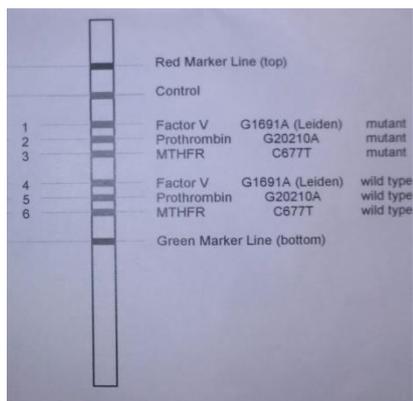
She was investigated for Lupus anticoagulant, measured by RVVT method according to the manufacturer's instructions and it was positive with screen time being 85 sec and confirms time 80 sec and the ratio of screen to confirm time was found to be above 1. Immunoglobulin G (IgG) anti cardiolipin antibodies were positive and showed values of 120 GPL (IgG phospholipid units) U/ml. IgM was also found to be positive with value 65 U/ml both of them measured by Enzyme Linked Immunosorbent Assay (ELISA) method. The patient was also tested for Vitamin D which was significantly low <3 ng/ml. Homocysteine level was 5.34ng/ml.

The patient was further investigated for the following genes in whole blood. The method adopted was Detection of C677T mutation in MTHFR gene, Prothrombin (Factor II) G20210A and FVG1691A (Leiden) mutation in Factor V Leiden based on polymerase chain reaction (PCR) and reverse hybridization. The results observed were MTHFR gene (C677T) positive (heterozygous) and Factor II (G20210A) and Factor V (FVG1691A) were not detected.

Later chromosomal analysis was done; the lymphocytes were cultured in PBMax medium with PHA stimulation for 72 hours. These cells are arrested using colcemid and the metaphases thus obtained were analyzed after GTG banding. Banding resolution is 500 (ISCN 2013) and 20 metaphases were analysed and karyotyped. The Karyotype of the patient was 46, XX, 21ps+ (female karyotype with increase in the length of

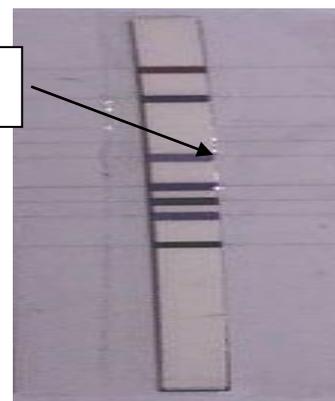
the satellite on short arm of chromosome 21).

Chromosomal analysis of the spouse was normal.

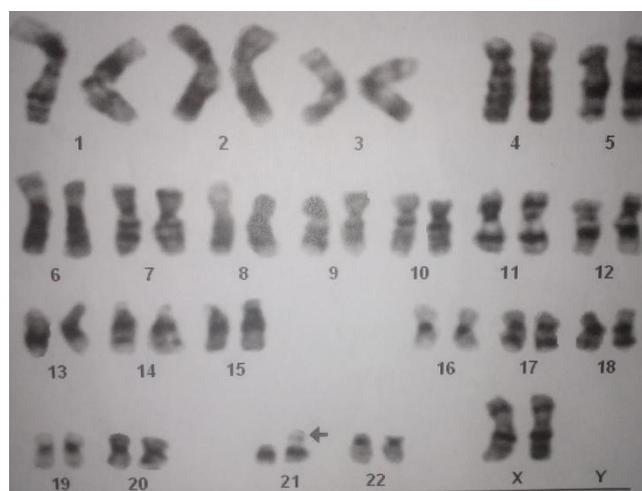


Control

**MTHFR
HETEROZYGOUS**



Patient



46, XX, 21ps+

DISCUSSION

Recurrent miscarriage refers to consecutive loss of 3 or more pregnancies. Antiphospholipid antibody syndrome is an autoimmune thrombophilic condition marked by the presence of antibodies in blood that recognize and attack phospholipid-binding proteins, rather than phospholipid itself. The clinical manifestations include vascular thrombosis and adverse pregnancy outcome like (a) consecutive miscarriages usually before 10 weeks of gestation.(b) one or more morphologically normal fetal losses after the 10th week of gestation. (c)one or more preterm births before the 34th week of gestation owing to placental disease.

The mechanisms by which antiphospholipid antibodies cause pregnancy morbidity includes inhibition of trophoblastic function and differentiation, activation of complement pathways at the maternal–fetal interface resulting in a local inflammatory response and in later pregnancy, thrombosis of the uteroplacental vasculature. Antiphospholipid antibodies are present in 15% of women with recurrent

miscarriage. In women with recurrent miscarriage associated with antiphospholipid antibodies; the live birth rate in pregnancies with no pharmacological intervention has been reported to be as low as 10% [4].

The C677T mutation in the methylene tetrahydrofolate reductase (MTHFR) gene leads to a reduction in the activity of this enzyme, resulting in increased levels of plasmatic homocysteine. Several series have suggested an association between hyperhomocysteinemia and RPL. Other gestational complications, such as preeclampsia, abruptio placentae, intrauterine growth restriction and stillbirth have also been reported [5].

Cellular immune abnormalities are increased in women with RPL and vitamin D deficiency. The target organs for the non classical actions of the vitamin D include innate and adaptive immune systems, pancreatic cells, heart, brain and reproductive tissues. Vitamin D has a role in hormonal regulation, modulation of immune responses and control of cellular

proliferation and differentiation. It inhibits the proliferation of T helper cells and decrease the production of cytokines like interleukin 2 and tumor necrosis factor alpha and interferon gamma. It has been observed that vitamin D could act as an immune regulator during implantation and play an important role in reproductive capacity. Studies have shown that vitamin D influences local anti-inflammatory response and induces decasualization of successful pregnancy [6].

Small structural chromosomal abnormalities like short tandem repeats, single nucleotide variants and copy number variants are known as polymorphisms. These generally are known to be responsible for most of the genetic variations in populations. Recent studies have mentioned the possible association of polymorphisms with reproductive failure and recurrent spontaneous miscarriages like Karyotype 46, XX, 21ps+ though no specific it is a type of polymorphism which is associated with sub fertility and also mental retardation [7].

The association between heterozygous C677T mutation in the MTHFR gene and ACL antibodies may increase the likelihood of thrombosis expression. It is known that persistently elevated serum levels of these antibodies are associated with RPL. Positive results for these antibodies maybe the starting point for the process of thrombosis. It is believed that this kind of association may be the key for understanding the mechanisms for several diseases, such as RPL. Studies have shown that the effect of antiphospholipid antibodies on trophoblast function and complement activation is reversed by heparin. In this case a low dose aspirin combined with heparin and high doses of folic acid can reduce morbidity and improves the pregnancy outcome.

CONCLUSION

Diagnosis requires a high index of suspicion when evaluating women with recurrent pregnancy loss. APL antibodies and heterozygous C677T mutation in the MTHFR gene presented a statistical association with RPL. The association of these two conditions is a new finding in thrombogenic factors for RPL, and may contribute to a greater understanding of this event.

REFERENCES

1. Andersen AMN, Wohlfahrt J, Christens P, Olsen J, Melbye M; Maternal age and fetal loss: population based register linkage study. *Bmj*, 2000; 320(7251):1708-1712.
2. Stephenson, M. D. (1996). Frequency of factors associated with habitual abortion in 197 couples. *Fertility and sterility*, 66(1), 24-29.
3. Di Prima FA, Valenti O, Hyseni E, Giorgio E, Faraci M, Renda E, Monte S; Antiphospholipid

Syndrome during pregnancy: the state of the art. *Journal of prenatal medicine*, 2011; 5(2):41.

4. Thiruchelvam A, Sekaran M; Thrombophilia abnormalities in recurrent pregnancy loss. *Biomedical Research-India*, 2010; 21(1):87-98.
5. Couto E, Barini R, Zaccaria R, Annicchino-Bizzacchi JM, Passini Junior R, Pereira BG, Silva JCGD; Association of anticardiolipin antibody and C677T in methylenetetrahydrofolate reductase mutation in women with recurrent spontaneous abortions: a new path to thrombophilia?. *Sao Paulo Medical Journal*, 2005;123(1):15-20.
6. Ota K, Dambaeva S, Han AR, Beaman K, Gilman-Sachs A, Kwak-Kim J; Vitamin D deficiency may be a risk factor for recurrent pregnancy losses by increasing cellular immunity and autoimmunity. *Hum Reprod*. 2014; 29(20): 208-219.
7. Madon PF, Athalye AS, Parikh FR; Polymorphic variants on chromosomes probably play a significant role in infertility. *Reproductive biomedicine online*, 2005; 11(6):726-732.