Case Report

Role of immunomodulation with lymphocyte immunization therapy (LIT) in a couple with 15 unexplained repeated miscarriages

Raut Mugdha M., Raut Mohan K.

Partners, Dr. Raut's Immunotherapy Centre for Prevention of Repeated Miscarriages (ICPRM), Mumbai

ABSTRACT

The problem of repeated miscarriages affects 0.5-2% of population. The current case is with history of 15 unexplained miscarriages following both, spontaneous conceptions and after IVF. All the routine investigations were normal. She had been treated with all the established methods of treatment without success. She was diagnosed with allo-immune cause of miscarriages and was given Lymphocyte Immunization Therapy (LIT). Her pregnancy successfully went till term. There were no side effects of the LIT to the mother or the baby. A case with higher number of repeated miscarriages is more likely to be due to allo-immune rejection of pregnancy. Immunomodulation with active immunotherapy in the form of LIT is still an effective therapy in properly selected cases.

Key Words: Allo-immunity, immunotherapy, lymphocyte immunization therapy (LIT), unexplained repeated miscarriages, unexplained recurrent miscarriages.

Received: 08 October 2018, **Accepted**: 16 October 2018

Corresponding Author: Mohan Raut, Immunotherapy Centre for Prevention of Repeated Miscarriages (ICPRM), Mumbai, India**, Email:** mohan@drraut.com.

ISSN: 2090-7265, February 2019, Vol.9, No.1

INTRODUCTION

The problem of repeated miscarriages is traumatic to a patient and is devastating when it happens repeatedly. The number of pregnancy losses is the accepted criterion in labeling a case as RPL. American Society of Reproductive Medicine(ASRM), considers two or more miscarriages as RPL. However, European Society of Human Reproduction and Embryology (ESHRE), uses three or more miscarriages for the definition of RPL[1]. With ASRM definition, incidents of RPL is 2%, while with ESHRE definition RPL affect .5-1% of pregnant women. As the number of miscarriages increases, it becomes increasingly difficult to treat as all the known tests to find the etiology are normal.

This case report discusses a case with 15 unexplained repeated miscarriages that resulted after spontaneous conceptions as well as after IVF treatment. All the known factors causing recurrent miscarriages were within normal limits. All the established modalities of treatment were unable to give success. In such cases, allo-immunity plays an important role and allo-immune rejection of pregnancy can explain the underlying cause. The case in discussion is unique in this matter, as the patient had experienced 15 miscarriages with failure of the established line of management and succeeded after immunomodulation treatment with lymphocyte immunization therapy (LIT).

Patient information

Thirty-six year old female residing out of India was presented with a history of 15 miscarriages. The couple was married for 10 years. All the miscarriages were confined to first trimester of pregnancy. The first eight miscarriages were following spontaneous conceptions. The next seven conceptions were following in vitro fertilization treatment. There was no history of any medical illness including diabetes mellitus, thyroid disorder or autoimmune disease. No history of smoking or alcohol addiction was there. There was no history of consanguinity and no family history of similar complaints. The patient had undergone all the established forms of treatment.

She was given progesterone, low dose of aspirin, low molecular weight heparin, vitamin supplements in the past pregnancies without success.

Clinical findings

Clinical examination of the patient was normal.

Diagnostic assessment

The couple had been investigated to rule out known factors of repeated miscarriages. Karyotype of both the partners was normal. Anatomical assessment of the uterus by ultrasonography and hysteroscopy was within normal limits. The tests for infections were normal. The

 DOI: 10.21608/EBWHJ.2019.28644

thrombophilia profile did not show any abnormality. Tests to rule out autoimmune disorders did not reveal any defect. The couple was investigated at our center for allo-immune incompatibility. The lymphocyte cross match test was negative [1+]. The peripheral blood natural killer cells were tested. CD3 was 78%, higher side of the normal range. Serum TNF alpha was within normal limits. The infection profile (HIV, HBsAg, HCV, VDRL) of both the partners was normal. Blood group of husband was A-positive and the wife was also A-positive. In view of the clinical history, negative lymphocyte cross match and a higher CD3 percentage, the couple was selected for LIT.

Therapeutic information

The couple was given LIT at our center (ICPRM). LIT is the active immunotherapy treatment used for couples with unexplained repeated miscarriages where all known factors for miscarriages are found to be normal and allo-immune factor is the likely cause. The couple underwent LIT with husband's lymphocytes. The paternal lymphocytes were separated by the process of centrifugation using special media. The separated lymphocytes were repeatedly washed to remove the paternal plasma and RBCs. The paternal lymphocyte suspension is injected into the wife by intradermal, subcutaneous and intravenous routes. The entire procedure of separation of the lymphocytes and injection of lymphocytes into the wife lasted for four hours. There was only one sitting of the procedure.

Follow up

After the procedure, the couple was observed for any reactions, which were none. The couple was asked to follow up after seven days to judge the response especially for the intradermal injections in the form of erythema and induration. No medications were given to the patient after the procedure. She was asked to avoid application of any cream, gel or ointment to the intradermal injection site for 24 hours. The patient was asked to report in case of any adverse reactions in the form of local and generalized pruritus, malaise, fever and breathlessness. The patient reported none of these. No tests were repeated after the procedure. The couple was asked to make attempts at conception after a period of four weeks from the time of therapy.

OUTOCOME

The couple went back to their country and planned pregnancy. The patient conceived spontaneously after two months [previous seven miscarriages were after IVF treatment]. The patient was advised to take progesterone, low dose of aspirin, low molecular weight heparin as soon as pregnancy was confirmed. Patient was on vitamins including folic acid. No booster dose of LIT was given. The pregnancy was followed up in her country. The pregnancy was uneventful. At 31 weeks, the fetus showed evidence of early IUGR for which she was given treatment. She delivered a male baby at 37 weeks [with birth weight of 2kg] by LSCS. The operation was uneventful. The child is two years old now, is healthy and with normal milestones.

DISCUSSION

The problem of repeated miscarriages is always a challenge to the treating physician. It is one of the most frustrating and difficult areas in reproductive medicine because the cause is many times unknown and diagnostic and therapeutic measures that are available are not always evidence based. The studies on the etiology, evaluation and the management of RPL are often flawed[2]. Hence, the treatment of Recurrent Pregnancy Loss (RPL) can be frustrating many times. The major challenge is the diagnosis of the etiological factors as all the established ones are within normal limits. In such cases, possibility of allo-immune problem is very high. It has been shown that, as the number of miscarriages increases, it is more likely that the chance of immunological problem is there. In such cases, immunomodulatorytreatment can play a very important role[3]. Of all the immunomodulatory treatments available, LIT presents an active form of treatment that can help to overcome this problem. LIT is a simple treatment, effective in properly selected patients, devoid of major side effects.

a) Literature

The earliest description of lymphocyte immunization was made by Billingham *et al*. They observed that skin grafts between fraternal twins were accepted, while grafts between non-twin siblings were not[4]. Inadequate maternal recognition of paternal allo-antigens could cause deficient tolerance to the pregnancy. Hence, it was reasoned that immunization with paternal mononuclear cells might enhance maternal recognition of paternal alloantigen, allowing patients suffering from recurrent and spontaneous abortion to carry a pregnancy to term[5]. In 1981, lymphocyte Immunotherapy (LIT) was performed to treat four URPL patients for the first time based on the “tolerance” of human kidney allografts, three delivered normal babies and one delivered a premature baby[6].

Mowbray *et al.* conducted the first randomized control trial of LIT in unexplained, recurrent, spontaneous abortion (RSA). 17 of 22 women given paternal cells had successful pregnancies compared with 10 of 27 given their own cells[7]. In 1994, a prospective collaborative study and meta-analysis confirmed the efficacy of LIT in women with primary recurrent spontaneous abortions without antipaternal antibobies but not in those with antipaternal antibodies[8].

A meta-analysis of all placebo-controlled trials showed that allogeneic lymphocyte transfusion (ALT) significantly increased the chance of live birth odds ratio 1.94 among patients with primary RM and no antipaternal antibodies[9]. A meta-analysis of women with five or more miscarriages who were given LIT was done. It showed that the chance

of live birth significantly increased following LIT.The beneficial effect was mainly seen in primary aborters[3].

b) Ober Study

In this study, immunization with paternal mononuclear cells did not improve pregnancy outcome in women with recurrent miscarriage. Despite a history of unexplained recurrent miscarriage, nearly 65% of control patients who became pregnant had a successful pregnancy. A higher rate of miscarriage was found in immunized women who became pregnant[10].

c) Criticism on Ober Study

The main criticism relates to the inclusion of the results by Ober *et al.*, the only study published to date that observed a negative effect of immunotherapy with lymphocytes on the rate of live birth. Ober study did not consider important factors like concentrate of paternal lymphocytes, storage of cells for several hours at a temperature between 1 and 6 °C, interval between the collection of the blood of the spouse and application of immunization, presence of autoimmune disorders, different immunotherapy administration routes (intradermal, subcutaneous and intravenous),number of doses and lymphocyte concentration[11].

d) Cochrane Review 2003, 2006, 2014 - Immunotherapy for recurrent miscarriages

Paternal cell immunization, third-party donor leukocytes, trophoblast membranes, and intravenous immunoglobulin provide no significant beneficial effect over placebo in improving the live birth rate.

e) Criticism of Cochrane Review

The mete-analysis in the Cochrane review itself has been criticized by many researchers due to various flaws associated with it. Many subsequent studies have pointed out the deficiencies in the Cochrane Meta-analysis (separate analysis of primary and secondary RM was not carried out, it did not distinguish between different immunizing doses and routes of administration, total number of miscarriages not considered) and have shown that actually Immunotherapy can play a beneficial role in recurrent spontaneous abortions and implantation failures in properly selected cases[12].

Liu *et al.* demonstrated that immunization with lymphocytes promoted a significant improvement in the rate of live births, 77.8% in the group of treated women, when compared with the rate of 46.1% in the control group, when Ober study was removed from the Cochrane meta-analysis[13].

In a new analysis, including the data by Ober *et al*. and by Stray-Pederson *et al*., which were excluded by Liu *et al*., the improvement in the rate of live births in couples who underwent immunotherapy (LIT) remained significant[14].

Rationale

In the current case, the role of allo-immune factor as the etiology appears the most certain. Here, we have ruled out all the known factors responsible for repeated miscarriages. The patient had already received various available modalities of treatment that are used for such patients but without any success. The lymphocyte cross match was negative; CD3 percentage was on the higher side of normal. The patient selection for LIT was done according to the following criteria:

1. History of 15 miscarriages.
2. First trimester miscarriages.
3. All routine investigations were normal.
4. Lymphocyte cross match was negative.
5. CD3 percentagerose.

Other criteria that are important are: (not present in this case)

6. Raised TNF alpha.
7. Presence of endometrial NK cells [CD57].

Primary takeaway lessons

A case with higher number of repeated miscarriages is more likely to be due to allo-immune rejection of pregnancy. If all the known factors for repeated miscarriages are ruled out, immunomodulation can help these patients to prevent subsequent miscarriage. Active immunotherapy in the form of LIT is still an effective therapy in properly selected cases.

Patient perspective

When the patient first approached, she was totally frustrated and demoralized by the problem. She had found about LIT by studying the literature on internet. She was willing to undergo any treatment that will help her without compromising her and her baby's health. She was willing to come to India just to undergo this treatment. She had found out about the treatment and already discussed with patients who had taken this treatment and had successful pregnancies. Now the patient is willing to share her experience with other patients suffering from similar problems who can be helped by LIT.

*Informed consen*t

Informed consent of the patient has been taken before the case study is prepared.

CONFLICT OF INTEREST

There are no conflicts of interest.

REFERENCES

1. American Society for Reproductive Medicine. Definitions of infertility and recurrent pregnancy loss.FertSteril 2008;90(Suppl 5): S60.

2. Christiansen OB, Nybo Andersen AM, Bosch E, *et al*. Evidence-based investigations and treatments or recurrent pregnancy loss. FertSteril 2005; 83(4): 821-39.

3. 3.Carp HJA, Toder V, TorchinskyA *et al.* Allogenic leucocyte immunization in women with five or more recurrent abortions. Hum Reprod 1997; 12: 250-5.

4. Billingham RE, Brent L, Medawar PB. 'Actively acquired tolerance of'foreign cells.1953. J Immunol 2010; 184: 5-8.

5. Beer AE, Semprini AE, Zhu XY etal.Pregnancy outcome inhuman couples with recurrent spontaneous abortions: HLA antigen profile; HLA antigen sharing; Female serum MLR blocking factors; Paternal leucocyte immunization. ExpClinImmunogenet 1985; 2: 137-53.

6. Taylor C, Faulk WP (1981) Prevention of recurrent abortion with leucocyte transfusions. Lancet 2: 68-70.

7. Mowbray JF, Gibbins C, Lidden H *et al.*Controlled trial of treatment ofrecurrent spontaneous abortion by immunization with paternal cells. Lancet 1985; 1(8435): 941-3.

8. Recurrent Miscarriages Immunotherapy Trialists Group (RTIMG). Worldwide Collaborative Observational study and meta-analysison allogenic leucocyte immunotherapyfor recurrent spontaneous abortion. Am J Reprod Immunol 1994; 32: 55-72.

9. Daya S, Gunby J. The effectiveness of allogenic leucocyte immunizationin unexplained primary recurrent spontaneous abortions.Am J ReprodImmunol 1994; 32: 294-302.

10. Ober C, Karrison T, Odem RB, Barnes RB, Branch DW,Stephenson MD Mononuclear-cell immunisation in prevention of recurrent miscarriages: a randomised trial. Lancet (1999); 354: 365–369.

11. Clark DA (2009) Cell-surface CD200 may predict efficacy ofpaternal mononuclear leukocyte immunotherapy in treatment ofhuman recurrent pregnancy loss. Am J ReprodImmunol 61: 75–84.

12. Christiansen O.Bwww.comtecmed.com/COGI/COGI9/Uploads/assets/christiansen%20ob.pdf.

13. Liu Z, Xu H, Kang X, Wang T, He L, Zhao A (2016) Allogeniclymphocyte immunotherapy for unexplained recurrent spontaneous abortion: a meta-analysis. Am J Reprod Immunol76: 443–453

14. Cavalcante MB, Sarno M, Edward AJ, Costa FDS, Barini R (2016) Arch GynaecolObstet DOI 10.1007/s00404-016-4270-z.