Lymphocyte Immunotherapy in Recurrent Pregnancy Loss: A Case Report*

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ABSTRACT

Recurrent Pregnancy Loss (RPL) is a multifactorial condition defined as having two or more consecutive pregnancy losses.[1,2,3] Investigation of the well- established causes is warranted to increase the chance of subsequent good pregnancy outcome. However, 50% of the RPL cases remains unexplained.[2,4,10] Pregnancy involves shift in the immunologic response to initiate immune tolerance.[6,7] Deviation from the normal immune tolerance response may lead to pregnancy failure and is the proposed mechanism of unexplained recurrent pregnancy loss.[13,14] Lymphocyte immunotherapy, which involves transfusion of paternal leukocytes to the maternal peripheral blood to induce an immune response creating a conducive environment for pregnancy maintenance, is a proposed therapy for unexplained RPL.[12,13]
This is a case report of a 40 year old, Gravida 7 Para 1, with Recurrent Pregnancy Loss, with no known genetic, uterine, and coagulation anomalies who initially underwent assisted reproductive techniques with Intrauterine Insemination and eventually underwent 4 cycles of Lymphocyte Immunotherapy, and was able to conceive and deliver at term.

Key words: recurrent pregnancy loss, lymphocyte immunotherapy, immune tolerance, alloimmune, pregnancy

Recurrent Pregnancy Loss (RPL) affects 1-2% of reproductive women.[1] It was traditionally defined as having three or more pregnancy losses of less than 20 weeks age of gestation or with a fetal weight of less than 500 grams.[2] Recent definition qualifies 2 or more pregnancy losses.[1,3] Exact pathologic process of RPL remains to be equivocal. About 50% of the RPL cases have identifiable risk factors or causes which include advanced maternal age, genetic abnormalities, maternal autoantibodies, endocrine dysfunctions, and uterine abnormalities. However, the remaining 50% is still unexplained.[2,4,10] Unexplained pregnancy loss was stipulated to be associated with immune dysregulation.[10] Thus, Lymphocyte Immunotherapy (LIT) is a proposed management for unexplained recurrent pregnancy loss. First documented to have a beneficial effect during the 1980s, LIT still had limited scientific evidence that supports it as a therapy for RPL.[12,14]

This is a case report on a 40 year old, Gravida 7 Para 1 (1061), with Recurrent Pregnancy Loss, who underwent Lymphocyte Immunotherapy for 4 cycles and was able to conceive and carry her pregnancy to term.

The objective of the study was to present a case of a 40 year old, Gravida 7 Para 1 (1061), with recurrent pregnancy loss and was treated with 4 cycles of lymphocyte immunotherapy.

THE CASE

This is a case of a 40 year old, Gravida 7 Para 1 (1061), with Recurrent Pregnancy Loss, who initially came in two years prior and is desirous of pregnancy. The patient and her husband have been trying to conceive since coitarche for 3 years now and had two failed pregnancies: an abortion, and an ectopic pregnancy, respectively. Controlled ovarian stimulation with Letrozole was initiated along with

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progesterone and folic acid which resulted in her third pregnancy. However, on approximately 8 weeks age of gestation, she had miscarriage and underwent completion curettage.

Still desirous of pregnancy, patient consulted another obstetrician where she underwent intrauterine insemination and was able to conceive for the fourth time, however, it resulted in another miscarriage at again approximately 8 weeks of gestation. Completion curettage was done. At that time, she had a total of 4 pregnancy losses, 3 of which were miscarriages and 1 ectopic. Laboratory work- ups were done such as karyotyping of the couple, APAS panel, and the husband's semen analysis which all revealed normal results. Transvaginal ultrasound revealed a normal sized anteverted uterus, multiple myoma uteri with M1 located in the right lateral area measuring 1.6 x 1.2 x 0.7 (FIGO 7), M2 located on the posterior area measuring 1.3 x 1.2 x 1.1 (FIGO 4), and M3 located on the posterior area measuring 0.8 x 0.9 x 0.4 (FIGO 4), with normal ovaries. Another session of intrauterine insemination was done and the patient had her fifth pregnancy, which, still resulted to abortion on her 8th week of gestation and patient again underwent completion curettage.

After five failed pregnancies, 4 spontaneous miscarriages and 1 ectopic pregnancy, referral to an Immunologist for initiation of lymphocyte immunotherapy (LIT) was suggested. A regimen of 3 cycles of LIT, probiotics, heparin with prednisone, folic acid, and micronized progesterone 200mg/cap, 1 cap three times a day per vagina were initiated. Patient underwent three cycles of LIT and spontaneously conceived thereafter resulting to her sixth pregnancy but resulted to another miscarriage approximately on the 8th week of gestation. Determined to be pregnant, the patient continued with the 4th cycle of LIT, which resulted in her 7th pregnancy. The same regimen of probiotics, heparin with prednisone, folic acid tablet once a day, and micronized progesterone three times a day were given. Pregnancy went beyond 8 weeks of gestation, reaching term without any complications. Patient delivered to a term, living, girl via Low Segment Cesarean Section at another institution. There were no postpartum and neonatal complications noted.

DISCUSSION

The author was presented with a 40-year-old, Gravida 7 Para 1 (1061) with poor obstetric history. Previous pregnancies were not carried beyond the first trimester, two of which were conceived through intrauterine insemination. She was diagnosed with Recurrent Pregnancy Loss. Referral to Immunologist for Lymphocyte Immunotherapy was done. After four cycles of LIT, she was able to conceive and had an unremarkable prenatal history and was able to deliver to a term, living, girl.

Pregnancy is an immunomodulation process wherein the maternal endometrium recognizes the embryo, a semi-allograft with both maternal and paternal genes, and generates an immunologic tolerance response to maintain pregnancy. On the other hand, failure to recognize the paternal antigens results to immune-mediated pregnancy rejection.[6,7] The exact mechanism of which is still investigated but several immunological adaptation theories have been postulated.[6,7,8] During pregnancy, there is shift in the Th1/Th2 paradigm; there is suppression of Th1 cytokines which is a pro-inflammatory, with subsequent upregulation of Th2 cytokines, an anti-inflammatory. Serologic shift of Th1/Th2 ratio favoring Th2 was noted to be pivotal in implantation and pregnancy maintenance.[6,13] Another population of T cells was recently discovered to be instrumental in pregnancy maintenance, T regulatory (Treg) cells. They are immune suppressing cells that prevent immunity against "self"-antigens.[20] These T cell populations are fundamental in establishing the pregnancy. It was hypothesized that changes in the Th1/Th2 ratio and the upregulation of the Treg cell population which indicates a shift to the humoral immunity and into an anti- inflammatory state are fundamental in pregnancy.[13,20] Human leukocyte antigen (HLA), another key component of maternal-fetal interface tolerance, is also crucial in the prevention of cytotoxic response of the maternal endometrium to the foreign embryo. HLA Class Ib is hypothesized to bind with the inhibitory receptor of the decidual natural killer cell allowing permissive invasion of trophoblast and establishment of maternal-fetal interface tolerance.[6,9,13] Furthermore, expression of the trophoblast of HLA-E, a subclass of HLA Class I antigen, is associated with preventing the uterine Natural Killer cells from attacking.[20] Discordance with the immune-tolerogenic response was hypothesized as a cause of unexplained recurrent pregnancy loss.[9]

Recurrent pregnancy loss has well-established causes such as uterine defects, obesity, hormonal diseases, and antiphospholipid syndrome. The remaining causes are yet to have robust evidence.[11]

Those whose causes are yet to be explained are categorized into unexplained recurrent pregnancy loss. The patient has been worked-up for causes of RPL after two consecutive non-induced pregnancy losses. The results of the APAS panel, thyroid function tests, karyotype, blood coagulation tests, and semen analysis of the partner were all normal. She was then advised to undergo intrauterine insemination and had two successful procedures but unfortunately resulted to miscarriage at approximately 8 weeks of gestation. To rule out uterine anatomic anomalies, transvaginal ultrasound was done which revealed multiple myoma uteri of intramural and subserosal in location. It has been suggested that intramural and submucous myomas may affect uterine receptivity which may contribute to pregnancy losses or implantation failure.[1] However, its direct causality is still yet to be established.[1,16] In intramural myomas that do not distort the uterine cavity, myomectomy may not be beneficial for patients who will undergo assisted reproductive techniques. Literature suggested that intramural myomas of >5.0 cm may benefit with myomectomy. Subserosal myomas do not affect outcome of assisted reproductive therapies.[16] In the present case, the authors were unable to identify the cause of recurrent pregnancy loss, thus an assessment of unexplained recurrent pregnancy loss was given.

Unexplained recurrent pregnancy loss was hypothesized to be associated with inappropriate immunologic response. Alloimmunization theories have been proposed among which is the Th1/Th2 hypothesis.[10,13,14] Th1, a pro-inflammatory cytokine, is known to be a threat to pregnancy maintenance. Increased levels have been implicated in RPL and multiple implantation failures.[13] Th2, on the other hand, is a cytokine responsible for allergic responses. Increase in Th2 is associated with pregnancy maintenance.[14] Apart from the Th1/Th2 hypothesis, the theory on human leukocyte antigen (HLA) compatibility of the couple was also introduced. It is the pioneer alloimmune theory wherein HLA class I molecules express genes that will facilitate immunotolerance.[9] Patient in the present case was requested with HLA Class I and HLA Class II compatibility tests which resulted in a negative cross-match reaction indicating an alloimmune dysfunction.

The inappropriate immune tolerance response is the principle used by Lymphocyte Immunotherapy as management of RPL. Its proposed mechanisms are the following: production of anti paternal cytotoxic antibodies, suppression of natural killer cell activity, and shift of the Th1/Th2 ratio favoring Th2 production.[9,13] The beneficial effect was first documented by Taylor and Faulk wherein 28 patients with RPL were given leukocyte-rich plasma from an unrelated donor which resulted in pregnancies in 20 of the participants and 81.5% of which had successful deliveries.[12,14,15] Basic protocol of LIT involves extraction of the partner's blood. Leukocytes are separated from the serum through centrifugation. Leukocytes then are washed out of the apheresis buffer. The solution then will undergo counterflow centrifugal elutriation which will separate lymphocytes by size. Monocytes, residual blood cells and platelets will then be removed from the solution. The remaining solution then will be injected to the patient.[12,13] One of its proposed mechanisms is it induces the production of antibodies which covers the paternal HLA in the fetal surface preventing the attack on the maternal natural killer cells.[1,13] A comparison of the lymphocyte population subsets before and after LIT showed significant difference among the production of IL-10, which is a cytokine of the Th2, and IFN- ɣ, which is a cytokine of Th1. There is a significant difference among the level of decrease in IFN-ɣ indicating a subsequent decrease in Th1 levels among those with successful pregnancies compared with those who suffered from miscarriage and underwent LIT.[21] It also shifts the Th1/Th2 ratio by reducing Th1, and increasing the Treg cells simulating the normal immune make-up of a normal pregnancy.[9] Several literatures have emerged on LIT as management of RPL. Patient consulted an Immunologist and was started with the LIT protocol, heparin and prednisone, micronized progesterone, folic acid, and probiotics administration. She underwent three cycles of LIT and conceived her 6th pregnancy but resulted to a miscarriage. Increase in maternal age is a consistent risk factor in abortion across several studies.[1,5,17] Furthermore, embryonic chromosomal anomalies have been implicated in 50% of early spontaneous abortion and are associated with increasing maternal age.[13,17,18] Women aged less than 35 years have 9%- 12% risk of sporadic abortion and the risk will increase significantly to 50% in women aged more than 40 years.[18] Apart from advanced maternal age, increasing number of previous abortions prior to treatment tend to have a higher chance of failure of LIT.[12,19]

Despite its lack of identifiable causative factor, the likelihood of a successful pregnancy in

unexplained recurrent pregnancy loss is still at 50-75%.[5] The patient underwent her 4th cycle of LIT and had her 7th pregnancy and was able to deliver to a live, term, baby girl with no noted neonatal complications. Presence of paternal antibodies in the patient's serum prior to pregnancy is a great predictor of a live birth in patients on LIT.[12] Women with primary recurrent pregnancy loss who underwent immunotherapy had better pregnancy outcome than those who had secondary recurrent pregnancy loss.[12]

One of the criticisms of LIT is the lack of standardized protocol and patient selection that would suffice a recommendation as treatment regimen for RPL.[8,9] Meta-analyses and review of studies on LIT efficacy have conflicting results. Furthermore, the Cochrane review of Immunotherapy on RPL emphasized the lack of statistically significant positive outcome of Immunotherapy.[8] The recent Guidelines on Recurrent Pregnancy Loss by the European Society of Human Reproduction and Embryology do not recommend LIT as a treatment of recurrent pregnancy loss due to its lack of credible and studies that support its proposed effect.[1] On the other hand, meta-analysis of LIT as treatment of RPL, increase in rate of live births was seen on patients who have been infused with fresh blood and is administered before and during pregnancy and if its administered through intradermal route.[8]

Recurrent pregnancy loss is a distressing condition that requires a long- term and holistic approach. The vague pathogenesis presents a diagnostic and therapeutic dilemma. With this, obstetricians should generate a management that is tailored to the individual.

CONCLUSION

In conclusion, recurrent pregnancy loss is investigated when two or more consecutive pregnancy losses are seen.[1,3] Despite the extensive laboratory work- up, 50% of the RPL cases remain unexplained.[11] Alloimmune dysregulation is thought to be the leading cause of unexplained RPL thus treatment offered to patients with unexplained RPL such as progesterone, aspirin, and low-dose folic acid.[1] Lymphocyte Immunotherapy also revolves on addressing the alloimmune dysregulation.[6,7,9] Conflicting data on LIT efficacy hinders its recommendation as a treatment for unexplained RPL.[8,9] Individualized investigation and management of RPL is still recommended.[1]

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