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ORIGINAL ARTICLE

Risk factors associated with a new pregnancy loss and perinatal outcomes in cases of recurrent miscarriage treated with lymphocyte immunotherapy

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Abstract

Objective: To assess the perinatal outcomes and risk factors for further pregnancy loss in patients with recurrent miscarriage treated with lymphocyte immunotherapy (LIT).
Methods: We performed a retrospective observational study of women with a history of two or more consecutive miscarriages who underwent LIT. All patients had undergone investigation of the etiology of the pregnancy losses according to a specific protocol. These etiologic factors were compared between those whose pregnancy outcome was successful and those who had a further miscarriage. The comparison between the groups was performed by Kruskal–Wallis, Fisher exact and Chi-square tests. Perinatal outcome data were collected for the successful pregnancies.
Results: One-hundred six patients were included. The mean number (±SD) of previous pregnancies, deliveries and miscarriages in all patients were 2.73 ± 0.8, 0.19 ± 0.4 and 2.54 ± 0.6, respectively. A successful pregnancy outcome after lymphocyte therapy occurred in 82 patients (group I), while 24 (22.6%) sustained a further miscarriage (group II). There was no statistical difference in the genetic, anatomic and hormonal causes of miscarriage between the groups ($p > 0.05$). Antinuclear (ANA) and antithyroglobulin (TgAb) autoantibodies occurred more frequently in group II ($p = 0.0010$ and $p = 0.0024$, respectively). Of those with successful pregnancies, 11 women (13.4%) had a preterm delivery. The mean birth weight was 3036.4 ± 498.6 g.
Conclusion: In patients with recurrent miscarriage treated with LIT, the presence of ANA and TgAb was a risk factor for further pregnancy loss. Perinatal outcomes in those whose pregnancies continued were favorable.

Keywords

Lymphocyte immunotherapy, miscarriage, perinatal outcome, recurrent miscarriage, risk factors

History

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Introduction

Recurrent miscarriage is an obstetric condition affecting about one to three percent of women, and has historically been defined as the loss of three or more consecutive pregnancies before 20 weeks of gestation with or without previous live births [1]. The American Society for Reproductive Medicine recently redefined recurrent pregnancy loss as two or more failed clinical pregnancies as documented by ultrasonography or histopathologic examination [2]. The pathophysiology of recurrent miscarriage remains incompletely understood, and as a result, the development of effective treatments for this condition continues to pose challenges. Genetic aberrations, uterine malformations and the antiphospholipid syndrome have already been defined as causes of recurrent miscarriage. Other factors that have been proposed as potential contributors to recurrent miscarriage include infectious diseases, activated autoimmunity, inherited thrombophilias and alloimmune mechanisms [1,3].

The embryo is considered to be an allograft by the maternal immune system. In 1966, Clark and Kirby [4] hypothesized that the disparity between maternal and fetal antigens may be beneficial for the development of the embryo. Maternal–fetal histocompatibility may induce a disturbance in the interaction between mother and fetus by preventing the maternal production of humoral and cellular factors essential to embryonic viability [5,6]. It has been noted that couples with several HLA alleles in common have increased rates of miscarriage [5,6]. One potential treatment option for these couples is lymphocyte immunotherapy (LIT).

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The therapeutic approach of administering partner or donor lymphocytes to women with recurrent miscarriage arose from the observation that patients undergoing repeated blood transfusions had a lower incidence of renal allograft rejection [7]. Decreased incidence of miscarriage in animals previously immunized with paternal spleen cells has also been observed [8]. Taylor and Faulk [9] were the first to report the use of non-paternal lymphocyte concentrate treatment for the prevention of recurrent miscarriage of unidentified cause. However, Beer et al. [5] proposed the use of the partner's lymphocytes as the preferred means of immunization.

The mechanisms that underpin LIT have not yet been fully elucidated. It is likely that this therapy promotes a favorable immune environment for embryo implantation through the formation of blocking antibodies [10] and interference with NK cell activity [11], the Th1/Th2 immunological response [12] and the balance of regulatory T lymphocytes [13].

Despite the controversy surrounding LIT, several centers worldwide have been employing it as a single treatment or in combination with other therapies in women with recurrent miscarriage. In this study, we analyzed the outcomes of a protocol for evaluation and treatment of couples with a history of recurrent miscarriage that includes LIT with or without other treatments and investigated risk factors associated with further miscarriage after immunotherapy.

Methods

This retrospective observational study was conducted by reviewing the medical records of 106 women with a history of two or more first trimester consecutive miscarriages who were treated with LIT using paternal lymphocytes between July 2010 and June 2012. Patients who underwent LIT demonstrated no antipaternal antibody production prior to treatment, and none had a prior history of infertility. All pregnancies included in this study were conceived spontaneously, without the assistance of artificial reproductive technology. All patients had undergone investigations for underlying causes of recurrent miscarriage according to a specific protocol and were divided into two groups according to the gestational result after LIT. Group I was composed of couples who had a successful pregnancy after this treatment, while group II consisted of couples who sustained a further miscarriage. Informed consent to administer immunotherapy was obtained from all participants, and the study was approved by the local ethics committee.

Protocol for evaluation and treatment

A genetic etiology for recurrent miscarriage was assessed through parental karyotyping. Hysterosalpingography and/or hysteroscopy were employed to assess for uterine anomalies as a cause of pregnancy loss. Thyroid function was assessed by free T4 and TSH measurement, and fasting glucose levels were used to determine the presence of diabetes mellitus. The acquired and inherited thrombophilias for which testing was performed were the antiphospholipid syndrome, protein C deficiency, protein S deficiency, antithrombin deficiency, methylenetetrahydrofolate reductase (MTHFR) C667T mutation, factor V Leiden and prothrombin gene G20210A mutation. Autoimmune factors were evaluated by testing for antinuclear (ANA), anti-DNA, thyroid peroxidase (TPOAb) and antithyroglobulin (TgAb) antibodies. All patients and partners underwent ABO and Rh blood group typing.

All patients underwent LIT, which was performed both before and during the first trimester of pregnancy. Progesterone was supplemented vaginally during the first trimester in all patients. Uterine malformations that could be corrected were surgically repaired prior to a further pregnancy. Couples with abnormal karyotypes received genetic counseling. Patients with any positive autoantibody result used prednisone (20 mg once a day) from the positive pregnancy test until 12 weeks of gestation. Patients with any positive test for thrombophilia (acquired or inherited) were treated throughout the pregnancy with low-dose aspirin from the first day of the last menstrual period and enoxaparin (40 mg once a day) from the positive pregnancy test onwards.

Lymphocyte immunotherapy

The protocol for LIT used in this study has been published previously [14]. Fresh blood (80 mL) was obtained from participants' partners by peripheral venepuncture and drawn directly into heparinized Vacutainer™ vials (Becton Dickinson & Co., Franklin Lakes, NJ). Peripheral mononuclear white blood cells (WBCs) were separated aseptically in laminar flow using Ficoll–Hypaque gradient centrifugation. WBCs were then washed in saline and resuspended in 1.0 mL of saline solution. Between 80 and 100 million cells were then administered to the forearm of the woman by intradermal injection in three locations. Immunizations were carried out on three different days, following the same routine, with a three-week interval between them. Three weeks after the last immunization, a crossmatch by complement-dependent cytotoxicity assay was performed to confirm antipaternal antibody production. To continue in the study, patients had to have a positive crossmatch after the initial three doses. Patients underwent booster immunization every three months while attempting pregnancy, and once every four weeks after a positive pregnancy test was achieved. All Rh-D negative patients received intramuscular anti-Rh D globulin (150 μg) immediately prior to the administration of paternal cells.

Statistical analysis

The characteristics of the study population have been presented as median or mean and range for continuous variables, and as discrete numbers and percentages for categorical variables. Comparisons between the groups were performed using the Kruskal–Wallis test, Fisher Exact Test and Chi-square. The data were transferred to an Excel 2007 spreadsheet (Microsoft Corp., Redmond, WA), and the statistical software package SPSS 20.0 (SPSS Inc., Chicago, IL) was used for statistical analysis. Differences were accepted as significant if $p < 0.05$.

Results

Baseline characteristics of participants are listed in Table 1. Patients with successful pregnancy outcomes following lymphocyte therapy were significantly younger than those

Table 1. Obstetric history of patients treated with lymphocyte immunotherapy.

	All patients ($n = 106$)		G-I ($n = 82$)		G-II ($n = 24$)		p
Age, years (mean, range)	32.0 ± 5.1	18–45	31.2 ± 5.1	18–45	35.0 ± 4.6	25–42	0.0028
Previous pregnancies (median, range)	2	2–7	2	2–7	2	2–4	0.4638
Parity (median, range)	0	0–2	0	0–2	0	0–1	0.3586
Miscarriage (median, range)	2	2–7	2	2–7	2	2–4	0.5650

G-I: couples who had a successful pregnancy after lymphocyte immunotherapy; G-II: couples who had a new miscarriage after lymphocyte immunotherapy; and p: G-I versus G-II.

who miscarried. There was no difference in obstetric history between the groups. Among the 106 patients, 86 (81.1%) had a history of primary recurrent miscarriage (no prior successful pregnancies).

An abnormal karyotype was observed in eight women (7.5%, 8/106), whereas all male karyotypes were normal. Eleven women of 106 (10.4%) had hypothyroidism controlled by use of thyroid hormone replacement at the time of pregnancy. The most common form of inherited thrombophilia was hetero- or homozygosity for the MTHFR C667T mutation (21.7%, 23/106). There was no statistical difference regarding the genetic, anatomic and hormonal potential causes of recurrent miscarriage between the groups (Table 2).

Among the autoantibodies tested, the most frequently identified were TPOAb (14.1%, 15/106). ANA and TgAb were more frequently present in women who miscarried than in those with successful pregnancies ($p = 0.0010$ and $p = 0.0024$, respectively) (Table 2). Among those who tested positive, mean serum levels of TgAb were significantly higher in the miscarriage group (125.83 IU/mL ± 116.31 IU/mL versus 72.60 IU/mL ± 37.38 IU/mL, $p = 0.037$). After correcting the values for maternal age, mean levels of TgAb remained elevated in the miscarriage group (128.90 IU/mL ± 65.68 IU/mL versus 71.733 IU/mL ± 63.64 IU/mL, $p < 0.001$). The miscarriage rate was 22.6% (24/106). Karyotyping on miscarried products of conception was performed in only seven cases (29.2%, 7/24). The karyotype was abnormal in three of these cases (42.9%), with trisomy 14 in two cases, and one case of monosomy X.

For women with successful pregnancies, the mean gestational age at delivery was 37.4 ± 1.6 weeks (range: 32–39 weeks). Full-term birth occurred in 71 of 82 deliveries (86.6%). Eleven pregnant women of 82 (13.4%) had a preterm delivery, four of which occurred before 34 weeks (36.4%, 4/11). The mean birth weight was 3036.4 ± 498.6 g (range: 1475–4050 g), and the mean birth length was 48.7 ± 2.4 cm (range: 42–58 cm).

Discussion

The effectiveness of LIT as a treatment for recurrent miscarriage remains controversial [15–18]. Although some authors have reported favorable outcomes following lymphocyte therapy [15,19], others consider this to be an ineffective treatment for recurrent miscarriage [16–18]. In 2004, Clark (a proponent of LIT) acknowledged the limitations of published trials in this area, including their small sample size, and inclusion of patients with ANA and karyotypically abnormal miscarriages [19]. Similarly, we observed that the ANA antibody was more frequently identified in those who had a further miscarriage following LIT. ANA positivity is a contraindication for this treatment in a Japanese protocol [20].

Table 2. Genetic, anatomic and hormonal cases of recurrent miscarriage in both groups treated with lymphocytes immunotherapy.

	Total of patients ($n = 106$)		G-I ($n = 82$)		G-II ($n = 24$)		
	%	n	%	n	%	n	p
Genetic	7.5	8	8.5	7	4.2	1	0.4207
Anatomic	11.3	12	8.5	7	20.8	5	0.0944
Hormonal	10.4	11	7.3	6	20.8	5	0.0691
APS	6.6	7	7.3	6	4.2	1	0.5008
PC	0	0	0	0	0	0	–
PS	5.7	6	6.1	5	4.2	1	0.5891
AT	1.9	2	2.4	2	0	0	0.5967
MTHFR	21.7	23	19.5	16	29.2	7	0.3128
FVL	0	0	0	0	0	0	–
PGM	1.9	2	1.2	1	4.2	1	0.4032
ANA	9.4	10	3.7	3	29.2	7	0.0010
aDNA	1.9	2	1.2	1	4.2	1	0.4032
TPOAb	14.1	15	12.2	10	20.8	5	0.2252
TgAb	10.4	11	4.9	4	29.2	7	0.0024

G-I: couples who had a successful pregnancy after lymphocyte immunotherapy; G-II: couples who had a new miscarriage after lymphocyte immunotherapy; APS: antiphospholipid syndrome; PC: protein C deficiency; PS: protein S deficiency; AT: antithrombin deficiency; MTHFR: methylenetetrahydrofolate reductase C667T mutation (heterozygous and homozygous); FVL: factor V Leiden (heterozygous and homozygous); PGM: prothrombin gene G20210A mutation (heterozygous and homozygous); ANA: antinuclear antibody; aDNA: anti-DNA; TPOAb: thyroid peroxidase antibody; and TgAb: antithyroglobulin antibody.

Although the precise pathophysiology remains unknown, an autoimmune-based etiology is one of several postulated causes for recurrent miscarriage and other adverse pregnancy outcomes. ANA and antithyroid antibody positivity seems to increase the risk of recurrent pregnancy loss and other gestational complications [21]. Iijima et al. have investigated the presence of autoantibodies in 1179 pregnant women and found a significantly higher rate of miscarriage in autoantibody positive patients, especially those with antithyroid microsomal (10.4%) or ANA (16.0%), compared with those who were antibody negative (5.5%) [22]. In a recent study that compared 160 women with recurrent miscarriage and 100 healthy controls, antithyroid autoantibodies were detected in 46 (28%) and 13 (13%) women, respectively ($p < 0.05$) [23]. In our study, ANA and TgAb were more frequent among women who miscarried. Our results are similar to those from other studies [21–25], although some authors disagree on the association between autoantibodies and recurrent miscarriage [26].

In this study, we demonstrated generally favorable perinatal outcomes in the group with ongoing pregnancies after LIT.

In 2004, Pandey et al. published a review of randomized LIT clinical trials, which demonstrated that women with recurrent miscarriage who had been treated with paternal LIT had more successful outcomes (68%) when compared to untreated women (54%, $p < 0.02$) [27]. However, when the results of the randomized and nonrandomized studies were pooled together, it was observed that 67% of women with RM from the study group who received paternal LIT showed successful pregnancy outcome in comparison to 36% success in women with RM from the control group ($p < 0.05$) [27].

In contrast to the findings of Pandey et al., the Cochrane review of immunotherapy for recurrent miscarriage, which was published in 2006 and included 12 high-quality prospective randomized trials of this treatment, did not identify a beneficial effect of immunotherapy over placebo [17]. The authors of this review conclude that further research is required to determine the potential benefit of such therapy, predicated on an improved understanding of the underlying pathophysiology of miscarriages of alloimmune origin.

Since publication of the Cochrane review, additional evidence in favor of immunotherapy has emerged. In a non-randomized, controlled trial by Gharesi-Fard et al., successful pregnancies occurred more frequently in a group of patients with recurrent miscarriage treated with LIT (67/92, 72.8%) in comparison to an untreated group (44/81, 54.3%). This study also reported a rate of miscarriage after LIT of 20/92 (27.2%), similar to our results of 24/106 (22.6%) [28]. Nonaka et al. have published results of a prospective non-randomized cohort study, in which 110 patients of 140 with recurrent miscarriage treated with LIT achieved a successful pregnancy (78.6%) [29]. In this study, the rate of successful pregnancies was significantly higher in patients treated with LIT and in those positive for mixed lymphocyte culture reaction – blocking antibodies (MLR-BAbs), a test of histocompatibility between the couple, than in non-immunized patients who were negative for MLR-BAbs [29].

In our study, full-term births occurred in 71 of 82 deliveries (86.6%), and there were thus 11 pre-term deliveries (13.4%). In the study by Nonaka et al., the rate of delivery post 36 weeks was 95.45% (105 of 110, including four small-for-gestational-age infants). Four infants were delivered prior to 36 weeks (3.64%), and one had a significant anomaly (gestation at delivery not recorded) [29]. The higher rate of preterm birth in our study can potentially be explained by different maternal characteristics between the populations studied [30].

Our study has limitations: it is a retrospective analysis and does not have a control group. Within a group of patients treated with LIT, however, we have demonstrated that the presence of ANA and TgAb are associated with an increased risk of further pregnancy loss.

More broadly, based on the data available in the literature, the involvement of auto- and alloimmune factors in the pathogenesis of recurrent miscarriage remains a challenge for researchers in this field. The controversies regarding immunotherapy in couples with recurrent miscarriage will only be resolved through further research in both the basic science and clinical domains. Prospective randomized controlled studies are needed to identify the optimal criteria for patient selection and to validate therapeutic protocols.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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