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Research Article

Studies on the Infants Born to the Patients with Unexplained Recurrent Abortion Who Underwent Immunotherapy using their Husband's Lymphocytes

Nonaka T[1], Takahashi M[1], Nonaka C[1], Enomoto T[1] and Takakuwa K[2]*

[1]Department of Obstetrics and Gynecology, Niigata University Medical and Dental Hospital, Japan

[2]General Center for Perinatal, Maternal and Neonatal Medicine, Niigata University Medical and Dental Hospital, Japan

***Corresponding author:** Koichi Takakuwa, General Center for Perinatal Maternal and Neonatal Medicine, Niigata University Medical and Dental Hospital, Japan

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Abstract

Purpose: To investigate the condition of infants born to the patients who underwent Immunotherapy with the Husband's Lymphocytes (LIT) for unexplained recurrent abortion.

Method: LIT was performed for the patients with unexplained recurrent abortion, and the pregnancy continued in 129 cases after LIT. The Gestational Weeks at Delivery (GW), the Body Weights (BW) of the infants, as well as major complications, were analyzed in these patients. As a control, the pregnancy outcome was analyzed in 243 cases with no complications and who did not receive LIT.

Results: Of 125 cases with singleton pregnancy (4 cases were excluded because of twins), the pregnancy terminated at term in 113 (90.4%), which was not significantly different from that in the control group (209/243, 86.0%, by the Chi-square test). The average GW and BW of infants in the study population and control group were 38W4D ± 2W0D and 38W4D ± 2W3D, 2968 ± 529g and 2904 ± 583g, respectively (not significant by non-paired t-test). There were also no significant differences in the rates of low birth weight and major complications between the two groups.

Conclusion: The outcome of pregnancies, especially the condition of infants, of patients after LIT was not significantly different from that of a control population.

Keywords: Unexplained recurrent abortion; Infants Immunotherapy; Husband's lymphocytes

Abbreviations

LIT: Immunotherapy with the Husband's Lymphocytes; GW: Gestational Weeks at Delivery; BW: Body Weight; MLR-BAbs: Mixed Lymphocyte Culture Reaction between Spouses; ≥22%: More than 22 Percent; GVH: Graft-Versus-Host Disease; RPL: Recurrent Pregnancy Loss; WG: Weeks of Gestation; LBWIs: Low-Birth-Weight Infants

Introduction

Although the etiology of recurrent spontaneous abortion, which is defined as three or more consecutive early pregnancy losses, is often unclear, several investigators have reported the existence of immunologically explainable recurrent spontaneous aborters [1-7]. Immunotherapy for these patients using their husbands' or a third party's leukocytes has been reported [7-25]. Although the efficacy of this modality has been controversial, an analysis of controlled clinical trials revealed the efficacy for patients with Unexplained Recurrent Abortion (URA) [7,14,19,26,27], especially for those who were negative for blocking antibodies evaluated by a mixed lymphocyte culture reaction between spouses (MLR-BAbs) [19,22,28-33]. The outcome of pregnancies, especially the condition of infants (such as the body weight of infants or the number of gestational weeks at which the infants were born) of patients after this immunotherapy, has not been fully elucidated, although some articles suggested absence of harmfulness of this immunotherapy for the infants born to immunized patients [34-39]. On the other hand, there is possibility that the above-mentioned MLR-BAbs, which are considered to be directed against paternal or fetal antigens, might adversely affect fetal growth. However, no reports have been published regarding the condition of infants born to the immunized patients in relationship with MLR-BAbs. In this context, we analyzed the outcome of pregnancies, especially the condition of infants, which had continued after immunotherapy using the husband's lymphocytes for patients with URA, in all of whom had been found to be positive for MLR-BAbs.

Materials and Methods

One hundred and sixty-two women with primary unexplained recurrent abortions were enrolled into current study between April 1983 and December 2012. The criteria for the immunotherapy were as follows: the patients consisted of primary habitual aborters who had experienced three or more consecutive first-trimester abortions; there is no apparent cause for the recurrent abortion (no muellerian anomaly, no chromosomal anomaly with couples, no hormone deficiency, no infectious diseases, no metabolic disorder and no

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autoimmune abnormalities such as positive anti-phospholipid antibodies); the patients are negative for MLR-BAbs as evaluated by a mixed lymphocyte reaction blocking assay.

Informed consent was obtained from all of the patients before the immunotherapy, and the protocol for the immunotherapy was approved by the Institutional Review Board of Niigata University School of Medicine.

Evaluation of MLR-BAbs

To evaluate the presence of MLR-BAbs, co-culture of mitomycin-C-treated paternal lymphocytes and untreated maternal lymphocytes was set up, complimented by control serum or with maternal autologous serum. The strength of blastoformation of the maternal lymphocytes was evaluated, and the MLR-blocking effect was calculated by the formula below. An MLR-blocking effect ≥22% was designated as positive for MLR-Babs [10,19,22,33].

MLR-blocking effect = (1 – mean cpm of culture in tested serum/ mean cpm of culture in AB serum) × 100 (%)

Immunotherapy protocol

Before the immunotherapy, the husbands were confirmed to be negative for syphilis, hepatitis viruses, HIV, and HTLV-1. Each husband's lymphocytes were obtained from approximately 100ml of heparinized blood, irradiated with 30Gy of X-rays to prevent any Graft-Versus-Host Disease (GVH) reaction, and suspended in 1ml of normal physiological saline solution. This cell suspension included lymphocytes, monocytes, and NK cells. After obtaining informed consent from the patient, the suspension was intradermally injected into the patients immediately after adjusting. Once MLR-BAbs had been positive in the patients' sera following a series of two or more vaccinations one month apart, the patients were encouraged to attempt to become pregnant [10-12,16,22].

The pregnancy continued in 129 cases after the immunotherapy. The Gestational Weeks of Delivery (GW), the Body Weight of infants (BW), and major complication of pregnancy were analyzed using medical records of the delivery. In case of delivery at another hospital, the records of the condition of the delivery and the infant were obtained via a patient questionnaire.

As a control group, the pregnancy outcome was analyzed in 243 pregnant cases who delivered infants in our hospital between January 2013 and December 2014. These patients did not have recurrent abortion and had not undergone the immunotherapy. Although some patients had a sporadic pregnancy loss before 10 weeks' gestation, they were no cases of Recurrent Pregnancy Loss (RPL), which was defined by 2 or more failed clinical pregnancies. Furthermore, some patients had a history of normal delivery. They were therefore diagnosed as having no medical complications at the early stage of pregnancy based on medical records detailing their history and medical checkups performed at the early stage of pregnancy, such as a complete blood count, blood sugar test, and tests for some infectious diseases. A series of ultrasonographic examinations done at the early stage of pregnancy revealed no abnormal findings in these control individuals.

Results

After undergoing immunotherapy with their husband's lymphocytes, all patients were allowed to attempt to become pregnant after the appearance of significant MLR-BAbs. Out of 162 patients who underwent the immunotherapy, the pregnancy successfully continued in 132, concluding in some manner in 129 and currently on-going in 3. Of the 129 concluded pregnancies, 4 were twins, and the rest (125 cases) were singletons. Table 1 shows the background data of the subject and control groups. Of the 125 cases of singleton pregnancy, the pregnancy terminated before 37 weeks of gestation (WG) in 12 cases (9.6%; 28WG in 1 case and 32-36WG in 11 cases) and at term in the remaining 113 cases. In the control group, the pregnancy terminated before 37 WG in 34 cases (14.0%) and at term in 209 cases. No significant difference was noted between the study population and the control population (chi-square test). The average GW in the study population and control group was 38W4D ± 2W0D and 38W4D ± 2W3D, respectively (not significant by non-paired t-test). The average BW of infants in the study population and control group was 2968 ± 529g and 2904 ± 583g, respectively (not significant by non-paired t-test). The frequency of low-birth-weight infants (LBWIs, <2500g) in the study population was 13.6% (17/125), while that in the control population was 9.47% (23/243) (not significantly different, Table 2). There were also no significant differences in the frequency of LBWIs between the two groups (Table 2). The mean total number of immunizations in 17 patients who delivered LBWIs was 2.294 ± 0.47, while that in the 243 patients who did not deliver LBWIs was 2.444 ± 0.60. There was no significant difference between these groups. In the study population the major anomaly of the infant (Corneria de Lange Syndrome) was observed in 1 case, there were no significant difference concerning the major complications between two groups.

Table 1: Background of patients group and control group.

	Patients with immunotherapy	Control
Number of individuals with singleton pregnancies	125	243
Mean age	30.5±4.6[a)]	32.2±4.1[a)]
Mean number of abortions	3.2±0.5[b)]	0.7±1.1[b)]
Mean number of deliveries	0[c)]	0.5±0.7[c)]

a) Not significant, b) P<0.0001 by non-paired T-test, c) P<0.001 by non-paired T-test.

Table 2: Pregnancy outcomes in patients group and control group.

	Patients with immunotherapy	Control
Rate of term delivery	90.4% (113/125)[a),b)]	86.0% (209/243)[b)]
Average gestational week of delivery	38W4D±2W0D [c)]	38W4D±2W3D [c)]
Average body weight of infants born at term	2968±529g [d)]	2904±583g [d)]
Rate of low birth weight infants(<2500g)	17/125 (13.6%)[e)]	23/243 (9.47%)[e)]

a) Excluding 4 cases of twin pregnancies, b) not significant by Chi-square test, c) not significant by non-paired T-test, d) not significant by non-paired T-test, e) not significant by Chi-square test.

Discussion

The outcome of pregnancies, especially the condition of the infants, in patients after immunotherapy with their husband's lymphocytes for URA, in all of whom significant MLR-BAbs had been observed to appear, was not significantly different from the outcome in the normal control population.

The efficacy of the immunotherapy has been controversial [8-23], but a meta-analysis concerning the immunotherapy for patients with URA reported by the Recurrent Miscarriage Immunotherapy Trialists Group in 1994 [13], Clark in 2008 [7] and 2012 [14] concluded and Liu et al. in 2016 [25] concluded that the immunotherapy may be highly effective. In addition, Pandey et al. reported a significant improvement in pregnancy outcome in the patient group in their double-blind randomized trial of husband's lymphocyte immunization for women with recurrent spontaneous abortion [19].

As one of the selection criteria for patients to be considered eligible for immunotherapy, the presence or absence of all immune factors before immunotherapy is considered to be important. Additional selection criteria include elevated blood NK cells and/or elevated Th1/Th2 cytokine levels in *in vitro*-activated PBL T cells, and absence of autoimmune features. In recent reports Liang et al. pointed out that NK cytotoxicity and Th1/Th2 ratios were significantly decreased in the successful conception group after the immunotherapy [24]. Furthermore, Khonina et al. suggested that the immunotherapy for patients negative for MLR-BAbs is accompanied by an increased proliferative cell response to the paternal alloantigens and enhanced production of soluble suppressor activity factors that is associated with improved pregnancy outcomes in women with URA [33].

Several investigators have reported the condition of infants born to patients with URA who had undergone immunotherapy with their husband's lymphocytes. Carp et al. reported that the rate of anomalies in infants was not significantly different between immunized and non-immunized patients [34]. Christiansen et al. [35] and Cavalcante et al. [36] indicated that the condition of infants born to mothers who had undergone the immunotherapy was not disturbed. We further found that the immunologic parameters were within the normal range in infants born to patients with URA who had undergone the immunotherapy in our serial follow-up study for the infants [37], and Malinowski et al. also reported no significant deterioration in the immunologic parameters of infants from patients with URA who had undergone immunization with paternal lymphocytes [38]. Moreover, Mowbray et al. reported that stronger boosting with paternal cells for patients with recurrent abortion might increase the body weight of infants [39]. All of these reports indicated lack of growth impairment effects on infants born to mothers who had undergone the immunotherapy. Nonetheless, there is the possibility that humoral factors, such as MLR-BAbs, induced by the immunotherapy with the husband's lymphocytes and directed against paternal or fetal antigens, exerted adverse effects on the growth of fetus of the immunized patients. However, most of the above-mentioned reports have not described the relationship between the appearance of such humoral factors and the condition of the infants born to the immunized patients.

In the current study, we analyzed the condition of infants who were born to patients who had undergone immunotherapy with husband's lymphocytes, in all of whom significant MLR-BAbs had been observed to appear, and compared with those who were born to pregnancy women with no complications. The average BW of infants born to treated patients (2968 ± 529g) was not significantly different from that in the control group (2904 ± 583g). Moreover, we clarified there were also no significant differences in the rates of low birth weight and major complications between the two groups. Although the frequency of LBWIs in the study population was slightly higher than that in the control population, the total number of immunizations was not significantly different between the patients who delivered LBWIs and those who did not. This indicates the lack of any adverse effects of immunization regarding the appearance of LBWIs.

In conclusion, immunotherapy using the husband's lymphocytes for patients with URA has no adverse effect on the infant's birth weight or the condition of infants, despite the presence of significant MLR-BAb.

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