Effectiveness of Leukocyte Immunotherapy in Primary Recurrent Spontaneous Abortion (RSA)

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ABSTRACT

Background: Recurrent spontaneous abortion (RSA) is defined as three or more sequential abortions before the twentieth week of gestation. There are evidences to support an allo-immunologic mechanism for RSA. One of the methods for treatment of RSA is leukocyte therapy; however there is still controversy about effectiveness of this method. **Objectives:** To evaluate the effectiveness of leukocyte therapy for treatment of RSA. **Methods:** Ninety two non-pregnant women with at least three sequential abortions (60 primary & 32 secondary aborters) recognized as RSA were referred to our Laboratory for immunotherapy. All the cases were immunized by isolated lymphocytes from their husbands. Fifty to 100 million washed and resuspended mononuclear cells were injected by I.V., S.C., and I.D. route. The result of each injection was checked by WBC cross matching between couples after four weeks of injections. Immunization was repeated in fifth week to a maximum of 3 times if needed. Eighty one age-matched non-pregnant RSA women (52 primary and 29 secondary aborters) with at least three sequential abortions were also included in this study as controls. The control group was not immunized. **Results:** 67 out of 92 (72.8%) immunized cases and 44 out of 81 controls (54.3%) showed a successful outcome of pregnancy ($p<0.02$). Comparison of primary and secondary aborters indicated a significantly better outcome only in primary (75% vs. 42.3%. $p<0.001$) but not in secondary aborters (68.8% vs. 75.9%, $p = 0.7$). **Conclusion:** The present investigation showed the effectiveness of leukocyte therapy in primary but not in secondary RSA patients. Despite the current controversy and limitation of leukocyte therapy in RSA, the results of our investigation provide evidence supporting the use of allo-immunization in improving the outcome of pregnancy in primary RSA patients.

Keywords: RSA, Leukocyte Immunization, Abortion

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INTRODUCTION

Habitual abortion is defined as three or more sequential abortions before the twentieth week of gestation from the last menstrual period or less than 500 grams of fetal body weight (1). In the vast majority of cases, the etiology is unknown (2, 3). Several factors such as genetic, anatomical, endocrine (4), and placental anomalies (5), or environmental (6) and immunological factors are being associated with a history of habitual abortions.It is thought that the immune reaction disorders in the mother may also contribute to the mechanism of RSA.

RSA can be classified into primary and secondary. The primary RSA patients are those who have aborted in all the previous pregnancies and have no live birth. Secondary RSA patients are those who have at least one successful pregnancy irrespective of the number of pregnancy losses. Certain evidence reveals allo-immunologic mechanisms for RSA (7). Pregnancy induced immune response is important for the maintenance of gestation and inadequate recognition of fetal antigens might lead to abortion in women with RSA (8). The effectiveness of blood transfusion in transplantation has previously been noticed. On the other hand several studies indicated an association between habitual abortion and sharing of human leukocytes antigens (HLA) with the father that may prohibit the production of anti paternal cytotoxic antibodies (APCA) in the mother during pregnancy (9, 10). Both allogenic leukocyte immunization and intravenous immunoglobulin (IVIg) therapy have been used for the treatment of women with unexplained recurrent spontaneous abortion (1, 11-13).

Efficiency of allogenic leukocyte immunization in the treatment of RSA was first demonstrated in a clinical trial by Mowbray in 1985 (14). In 1994, a meta-analysis of all placebo-controlled trials showed that allogeneic leukocyte therapy significantly increased the chance of live birth among patients with primary RSA (15).The use of allogenic lymphocyte transfusion became a quite widespread and accepted treatment until 1999 at which time the results of a large placebo-controlled trial showed that immunotherapy did not increase the chance of live birth compared to placebo but rather tended to decrease it (16). Following the work of Ober et al (16), several other meta analyses also indicated that leukocyte therapy has no effect in improving the success rate in RSA cases (17, 18). On the other hand there are several recent studies that indicate the benefit of this method for the treatment of a group of RSA patients (1, 11, 19).

Leukocyte immunization is now employed to treat a subgroup of RSA women; however its effectiveness continues to be controversial. The present study was undertaken in a group of women with RSA at our center to evaluate the effectiveness of lymphocyte immunization in Iranian women with primary RSA.

MATERIALS AND METHODS

Subjects. Ninety two non-pregnant women between 22 and 38 years of age with at least three sequential abortions with the same partner referring to our center during December 2000 to September 2005 formed the study cases. Among the cases, 60 were primary and the remaining 32 were secondary aborters.

Eighty one age and ethnic matched non-pregnant women with at least three sequential abortions were also included as controls. Among our control group, 52 were primary and 29 were secondary aborters. Informed consent, approved by local ethics committee,

was obtained from all cases and controls. The cases and controls were investigated clinically and serologically by a gynecologist and were diagnosed as RSA. No endocrine anomaly and no evidence of anti-nuclear and anti-phospholipids syndrome was detected. All the patients had negative WBC cross match with their husbands before therapy (checked by a serological method). The patients were checked for the presence of blood-borne infectious diseases such as HIV and hepatitis B and C. ABO blood grouping was also checked to prevent transferring of Rhesus factor (Rh) positive RBC to Rh negative patients.

Immunotherapy. Immunization with isolated lymphocytes from their partners by the following procedure was performed in all of the cases. Mononuclear cells were separated using Ficoll-Hypaque gradient. Washed and resuspended cells in 3 ml of sterile saline were aspirated into 3 syringes, one having 2.0 ml and the other two having 0.5 ml of the cell suspension .The total number of cells used were approximately 50-100 million cells. The 2.0 ml suspension was given by IV drip and the two 0.5 ml suspensions were administered, into the forearm S.C and I.D, respectively. The results of immunization were checked after four weeks by WBC cross matching between couples using a serological method. Immunization was repeated in the fifth week to a maximum of 3 times if WBC cross-match showed less than 20% reactivity. Furthermore, immunized women were evaluated for WBC cross-match reactivity in the first trimester of pregnancy and if the test showed less than 20% reactivity, a booster immunization was done with the same protocol. Control groups were not immunized**.** Treatment success was defined as a pregnancy with live birth and treatment failure was defined as a pregnancy failure before 20 weeks of gestation.

Statistical Analysis. Statistical analysis were carried out using (SPSS), version 11.5 for Windows. Chi-square test with Yates correction was used for comparison of cases and controls. P-values less than 0.05 were considered as significant. All reported P-values were two-tailed.

RESULTS

Figure 1 shows the pregnancy outcome of patients and RSA controls after immunization. Total success rate in subjects with a completed immunization protocol was 72.8%. Sixty seven out of ninety two cases (72.8%) became pregnant successfully and gave birth while twenty five of our patients (27.2%) experienced another abortion after therapy. All the children born were normal. In addition in non immunized group, 54.3% became successfully pregnant while the remaining 37 (45.7%) experienced another abortion.

Statistical analysis indicated a significant difference in success rate of pregnancy outcome between cases and controls (72.8% vs. 54.3% $p<0.02$) (Figure 1).

Outcome of pregnancy was evaluated and compared in immunized and non-immunized cases according to primary or secondary abortions.

Forty five out of sixty primary aborters (75%) became pregnant successfully while in our non-immunized group, the success rate was 42.3% ($p<0.001$) (Table1). There was no significant difference between immunized and non-immunized secondary aborters with respect to pregnancy outcome (68.8% vs. 75.9%, $p=0.7$) (Table1).

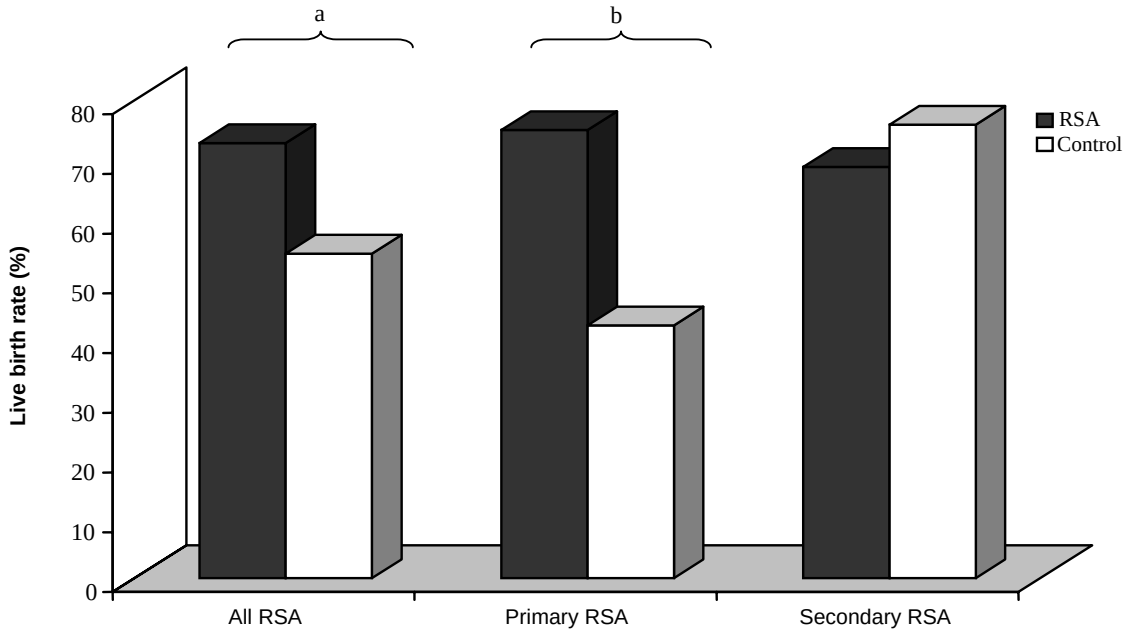

Figure 1. Live birth rates of immunized and non-immunized RSA patients. [a] P < 0.02, [b] P < 0.001

Table 1. Outcome of pregnancies in primary and secondary RSA patients and their corresponding controls

Outcome	RSA (n = 92)		Controls (n = 81)	
	Primary[a] (n =60)	Secondary[b] (n=32)	Primary(n =52)	Secondary(n=29)
Successful pregnancy	45 (75%)	22 (68.8%)	22 (42.3%)	22 (75.9%)
Abortion	15 (25%)	10 (31.2%)	30 (57.7%)	7 (24.1%)

[a]p < 0.001, Primary versus primary aborter.
[b]p =0.7, Secondary versus secondary aborter.

DISCUSSION

In this study, we investigated the effect of paternal immunotherapy on the outcome of pregnancy in patients with unexplained RSA, and also compared the results between primary and secondary aborters.

Many recent studies or meta analysis have been published against advantages of leukocyte therapy (17, 18, 20). There are also several studies demonstrating that immunization with paternal lymphocytes attributes to the success of pregnancy in women with RSA (1, 11, 19, 21).

In the present study we registered 92 non-pregnant women with at least 3 sequential abortions for immunotherapy and compared the effect of this treatment in different groups of patients including primary and secondary aborters, where immunization was performed before and during pregnancy if required.

Our results revealed that 72.8% of immunized women showed a significantly successful outcome of pregnancy while our non-immunized group, showed 54.3% success rate in pregnancy (p< 0.02). Upon comparison of various updated randomized trials of paternal

lymphocyte immunotherapy for women with RSA (21, 22, 23, 24), we found a higher pregnancy outcome in our immunized group (72.8%).
However Ober et al (16) reported that this mode of therapeutic approach does not improve pregnancy outcome in women with RSA, but they considered those cases that did not get pregnant 12 months after immunization beside those who experienced another abortion before 28 weeks of gestation as treatment failure. On the other hand Ober did not compare primary and secondary aborters which may affect the results.
There are also two computer data-based meta analysis reports by Scott (17) and Porter (18) indicating that leukocyte therapy has no therapeutic effect for RSA patients.
The last two published meta analysis which give no support for leukocyte therapy in RSA patients, extracted data from laboratories with heterogeneous patients and with variations in protocols used. In addition, one can consider other factors affecting the results of their trials, among which one could mention the numbers of previous miscarriages, presence of prior live birth, time of conception after immunization and the number of injected cells and the time used for immunization. The major drawbacks to such studies are heterogeneity of patients and variations in criteria used for patient selection and the different backgrounds of the studied group and also differences in treatment protocols.
Published reports on pregnancy outcome with primary versus secondary abortions are very limited. In one report by Christiansen et al , the results of the placebo-controlled trials carried out in the Danish recurrent miscarriage clinic, indicated that allogenic lymphocyte transfusion improved live birth in women with primary recurrent miscarriage while IVIG improved live birth rate in those with secondary recurrent miscarriage and IFD (1). Consistent with Christiansen data, we showed that paternal leukocyte therapy clearly improved pregnancy outcome in primary RSA (75% vs. 42.3%, $p< 0.001$).
The effect of immunotherapy as a therapeutic approach for women with unexplained recurrent miscarriage is still a challenging issue due to several factors:
(1) Most of the immunotherapy trials are too heterogeneous with respect to the patients and the treatment protocols; (2) Differences in immunization protocols that is the total number of cells and the time of immunization ; (3) Certain factors such as the number of previous miscarriages, presence of prior live births, patient's age and time of conception after immunization.
Finding a significant association between lymphocyte immunotherapy and successful pregnancy emphasizes that immunotherapy given correctly to right patients may have therapeutic benefits.

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REFERENCES

1 Christiansen OB, Nielsen HS, Pedersen B. Active or passive immunization in unexplained recurrent miscarriage. J Reprod Immunol. 2004; 62: 41-52.
2 Stern JJ, Dorfmann AD, Gutierrez-Najar AJ, Cerrillo M, Coulam CB. Frequency of abnormal karyotypes among abortuses from women with and without a history of recurrent spontaneous abortion. Fertil Steril. 1996; 65: 250-3.
3 Ohno M, Maeda T, Matsunobu A. A cytogenetic study of spontaneous abortions with direct analysis of chorionic villi. Obstet Gynecol. 1991; 77:394-8.

4 Li TC, Makris M, Tomsu M, Tuckerman E, Laird S.Recurrent miscarriage: aetiology, management and prognosis. Hum Reprod Update. 2002; 8:463-81.
5 Torpin R. Placenta circumvallata and placenta marginata. Obstet Gynecol. 1995; 21: 76-81.
6 Gardella JR, Hill JA 3rd. Environmental toxins associated with recurrent pregnancy loss. Semin Reprod Med. 2000; 18:407-24.
7 Porter Tf, Scott JR. Alloimmune causes of recurrent pregnancy loss. Semin Reprod Med. 2000; 18:393-400.
8 Larid SM, Tuckerman EM, Cork BA, LinjaWi S, Blakemore AI, Li TC.A review of immune cells and molecules in women with recurrent miscarriage. Hum Reprod Update. 2003; 9:163-74.
9 Beydoun H, Saftlas AF. Association of human leucocyte antigen sharing with recurrent spontaneous abortions. Tissue Antigens. 2005; 65: 123-35.
10 Kishore R, Agarwal S, Halder A, Das V, Shukla BR, Agrawal SS. HLA sharing, anti-paternal cytotoxic antibodies and MLR blocking factors in women with recurrent spontaneous abortion. J Obstet Gynaecol Res.1996; 22:177-83.
11 Pandey MK, Thakur S, Agrawal S. Lymphocyte immunotherapy and its probable mechanism in the maintenance of pregnancy in women with recurrent spontaneous abortion. Arch Gynecol Obstet. 2004; 269:161-72.
12 Adachi H, Takakuwa K, Mitsui T, Ishii K, Tamura M, Tanaka K. Results of immunotherapy for patients with unexplained secondary abortions. Clin Immunol. 2003; 106:175-80.
13 Ramhorst R, Agriello E, Zittermann S, Pando M, Larriba J, Irigoyen M et al. Is the paternal mononuclear cells immunization a successful treatment for recurrent spontaneous abortion. Am J Reprod Immunol.2000; 44: 129 -35.
14 Mowbray JF, Gibbings C, Liddell H, Reginald PW, Underwood JL, Beard RW. Controlled trial of treatment of recurrent spontaneous abortion by immunization with paternal cells. Lancet. 1985; 1; 941-3.
15 Daya S, Gunby J. The effectiveness of allogeneic leukocyte immunization in unexplained primary recurrent spontaneous abortion. Recurrent Miscarriage Immunotherapy Trialists Group.. Am J Reprod Immunol. 1994; 32: 294-302.
16 Ober C, Karrison T, Odem RR, Barnes RB, Branch DW, Stephenson MD et al. Mononuclear-cell immunisation in prevention of recurrent miscarriages: a randomised trial.. Lancet. 1999; 354: 365-9.
17 Scott JR, Immunotherapy for recurrent miscarriage. Cochrane Database Syst Rev. 2003;1:CD000112.
18 Porter TF, LaCoursiere Y, Scott JR. Immunotherapy for recurrent miscarriage. Cochrane Database Syst Rev. 2006; 2:CD000112.
19 Chaichian S, Shoaee S, Saremi A, Pedar S, Firouzi F. Factors influencing success rate of leukocyte immunization and anti-paternal antibodies in spontaneous recurrent miscarriage.. Am J Reprod Immunol. 2007; 57:169- 76.
20 Chaouat G. Should we re-examine the status of lymphocyte alloimmunization therapy for recurrent spontaneous abortion? Am J Reprod Immunol.2003; 50:433-8.
21 Pandey MK, Agrawal S. Induction of MLR-Bf and protection of fetal loss: a current double blind randomized trial of paternal lymphocyte immunization for women with recurrent spontaneous abortion. Int Immunopharmacol. 2004; 4:289-98.
22 Li D, Li C, Zhu Y. Comparative study of the third party and paternal leukocyte immunization in recurrent spontaneous abortion of lowered maternal-fetal immuno-recognition. Zhonghua Fu Chan Ke Za Zhi. 1998; 33: 597-600.
23 Ramhorst R, Agriello E, Zittermann S, Pando M, Larriba J, Irigoyen M et al. Is the paternal mononuclear cells' immunization a successful treatment for recurrent spontaneous abortion? Am J Reprod Immunol. 2000; 44:129-35.
24 Carp HJ, Toder V, Torchinsky A, Portuguese S, Lipitz S, Gazit E et al. Allogenic leukocyte immunization after five or more miscarriages. Recurrent Miscarriage Immunotherapy Trialists Group.. Hum Reprod 1997; 12:250-5.