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Soluble CD30 (sCD30) and effectiveness of leukocyte therapy in recurrent pregnancy loss patients

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ABSTRACT

While there is still controversy regarding its effectiveness, leukocyte therapy is considered a useful method of treatment for a subgroup of patients with recurrent pregnancy loss (RPL). To date, there is no reliable test for evaluating the effectiveness of leukocyte therapy. Deviation in the cytokine balance toward a Th2 response is suggested to be the major mechanism underlying the benefit of leukocyte therapy. sCD30 is an index of activity of the Th2 arm of the immune system. The aim of this study was to determine the effect of leukocyte therapy on serum sCD30 and also to investigate the relationship between the sCD30 level after therapy and pregnancy outcome. The sCD30 titer in a group of RPL patients treated with paternal leukocytes was measured before and after therapy and compared with two control groups (normal non-pregnant and first trimester pregnant women). The mean level of sCD30 was found to be significantly increased after treatment. A significant increase in the mean level of sCD30 was observed in patients after leukocyte therapy irrespective of later successful or unsuccessful pregnancy outcome. No correlation between the increase in sCD30 level and pregnancy outcome was observed. In conclusion, the results of the present study show that leukocyte therapy increases the sCD30 level; however, the rise in the sCD30 level is not correlated with a successful outcome.

1. Introduction

Miscarriage is a common phenomenon complicating more than half of pregnancies (Sierra and Stephenson, 2006). Three or more consecutive pregnancy losses, before the twentieth week of gestation, is defined as recurrent pregnancy loss (RPL) and this occurs in 1–2 percent of all pregnancies (Practice Committee of the American Society for Reproductive Medicine, 2008). Several etiological factors such as chromosomal, placental anomalies, hormonal, anatomical, endocrine and immunological factors are proposed to be associated with a history of RPL (Quenby et al., 2002; Regan et al., 2011). When the etiology of abortion is unknown, habitual abortion is called unexplained RPL (URPL) (Stephenson, 1996). It is widely believed that an abnormality in the maternal immune reaction to the fetus is the main reason for URPL. During pregnancy, fetal antigens are in close contact with cells of the maternal immune system and it is believed that cytokines are important mediating agents through which maternal immune cells communicate in response to the semi-allogenic placental antigens (Van Mourik et al., 2009). Cytokines of the Th1 type are shown to be deleterious to pregnancy, while those of the Th2 type act in favor of a successful pregnancy (Raghupathy, 1997). In parallel with the Th1/Th2 hypothesis, the dominant theory indicates that the fetus induces the Th0 cells of the immune system to choose a Th2 pathway for

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maturation, which subsequently contributes to down regulation of the deleterious Th1 arm of the immune response (Saito et al., 1999). It is believed that RPL is characterized by exaggerated Th1 responses. Nevertheless, there is still debate on the credibility of this theory. For example, in some RPL cases the Th2 arm is dominant (Bates et al., 2002; Chaouat et al., 2003).

Activated T cells of the Th2 phenotype secrete CD30 (Romagnani et al., 1995). CD30, a glycoprotein receptor expressed by Th2 cells, is a member of the tumor necrosis factor receptor superfamily that modulates the Th1/Th2 balance (Smith et al., 1993). The soluble form of CD30 (sCD30) is produced by proteolytic cleavage of the extracellular domain of CD30 (Hansen et al., 1995). sCD30 has been shown to be a serum index of Th2 immune responses (Hoshimoto et al., 2000; Romagnani et al., 1995). The role of sCD30 in pregnancy is accentuated by findings that firstly, sCD30 increases significantly in the serum of women as they become pregnant, and secondly, patients with pregnancy related disorders such as preeclampsia as well as those who will have a small for gestational age neonate have drastically lower circulating sCD30 compared with normal pregnant women (Kusanovic et al., 2007a,b; Laskowska et al., 2010).

Paternal leukocyte therapy is one the methods used for treatment of RPL. While there is still controversy about its effectiveness, leukocyte therapy is used for treatment of a subgroup of RPL women (Chaichian et al., 2007; Clark et al., 1996, 2001; Clark, 2004, 2008; Coulam and Acacio, 2012; Gharesi-Fard et al., 2007; Ford and Schust, 2009; Lin and Qiu, 2010; Pandey et al., 2004; Porter et al., 2006; Scott, 2003). Heterogeneity in immunization protocols and inclusion criteria for selection of candidate women both account for divergent results when the efficacy of leukocyte therapy is investigated.

To date there is no reliable test for evaluating the results of leukocyte therapy. Deviation in the cytokine balance is suggested to be a major mechanism underlying the benefit of leukocyte therapy. In this respect, Yokoo et al. reported that deviation of Th1 toward Th2 cytokines is related to a positive outcome of leukocyte therapy (Yokoo et al., 2006). We also reported a significant decrease in the level of TNFα in sera of women who had a successful pregnancy outcome after leukocyte therapy (Gharesi-Fard et al., 2008). The aim of the present study was the investigation of the correlation between serum sCD30 level, as a Th2 index, and effectiveness of leukocyte therapy in URPL women. To fulfill this goal, the level of sCD30 was measured in serum recovered before and after leukocyte therapy in a group of Iranian URPL cases who have successful outcome and compared with URPL cases with unsuccessful outcome after leukocyte therapy.

2. Materials and methods

2.1. Subjects

Thirty URPL women between 27 and 37 years of age (31.4 ± 3.1) with at least three previous abortions and two years follow up after therapy formed the subjects of the present study. Based on the pregnancy outcome patients were divided into two different groups. Of a total of 30 cases, 15 labeled as group A had a positive outcome (live birth after therapy) while the remaining 15, group B, showed a negative outcome (another pregnancy failure before 20 weeks of gestation) after therapy. All cases were primary aborters without any history of previous live delivery or infertility. The diagnosis of URPL was based on clinical and laboratory findings. All URPL patients were evaluated for normal clinical criteria by a gynecologist and laboratory test to rule out the presence of infections (toxoplasma, cytomegalovirus, rubella, HIV, Chlamydia, hepatitis B and C) and anti-phospholipid antibodies (including lupus anticoagulant, anti-cardiolipin and β2-glycoprotein antibodies) and also anti-thyroid antibodies. All patients and their previous aborted fetus had a normal karyotype pattern report. Moreover we included two additional groups of women as controls in this study. Group C consisted of 43 normal non-pregnant women without any history of pregnancy and group D were 42 normal pregnant women at first trimester (gestational week 8–12), with a history of at least two previous pregnancies and without any history of abortion. All participating groups in this study were matched by age. Informed consent was obtained from all participants and this study was approved by the local ethics committee of the Shiraz University of Medical Sciences.

2.2. Immunotherapy

All patients received lymphocytes from their partners for the leukocyte therapy according to the same protocol as previously described (Gharesi-Fard et al., 2008). Before therapy, using a serological method, all women were shown to be negative for anti-paternal mononuclear cell antibodies (checked by WBC cross match with their husbands leukocytes). A maximum of three rounds of leukocyte therapy (with four week interval between each injection) were performed until the WBC cross matching with partner showed a positive result.

2.3. Soluble CD30 measurement

Before and after therapy 2 ml of peripheral blood was collected from patients and samples were centrifuged at $1000 \times g$ for 10 min to obtain serum. Sera were frozen at $-70\,^{\circ}C$ until sCD30 measurement. An ELISA technique was used for evaluating the sCD30 level (Bender Med Systems, Vienna, Austria). The detection range of the ELISA kit was 0.33–100 ng/ml.

2.4. Statistical analysis

To perform statistical analysis on our data we used SPSS version 17 software for Windows (SPSS Inc., Chicago, IL, USA). After considering the number of cases, for comparison of the sCD30 mean levels between groups, the Mann–Whitney test (exact, two-tailed) was used and p values less than 0.05 were considered significant.