Role of inflammatory mediators in patients with recurrent pregnancy loss

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Objective: To examine interleukin-12 (IL-12), IL-18, IFN-γ, intracellular adhesion molecule-1 (ICAM-1), leukemia inhibitory factor (LIF), and migration inhibitory factor (MIF) levels in precisely-timed blood and endometrial tissue samples from women with idiopathic recurrent pregnancy loss (RPL).
Design: Case-control study.
Setting: University hospital.
Patient(s): Twenty-one women with RPL and 20 women with proven fertility (controls).
Intervention(s): Primary endometrial cells and blood samples during the midsecretory phase (days 19–23).
Main Outcome Measure(s): Detection of IL-12, IL-18, IFN-γ, ICAM-1, LIF, and MIF via enzyme-linked immunosorbent assay in both blood and endometrial tissue samples.
Result(s): The blood and tissue levels of IL-12, IL-18, and IFN-γ were statistically significantly higher, and the blood and tissue levels of LIF and MIF were statistically significantly lower in patients with RPL. Only the level of tissue ICAM-1 was higher in patients with RPL. There was a strong correlation between blood and tissue level measurements of IL-12, IL-18, LIF, and MIF.
Conclusion(s): Our findings support the hypothesis that inflammatory processes may contribute to pregnancy loss, possibly through their role in implantation. We found that blood and tissue levels of IL-18, LIF, and MIF, and tissue levels of IL-12, IFN-γ, and ICAM-1 have statistically significant prognostic relevance. (Fertil Steril® 2015;■:■–■. ©2015 by American Society for Reproductive Medicine.)
Key Words: IL-12, IL-18, interferon-γ, intercellular adhesion molecule-1, leukemia inhibitory factor, macrophage migration inhibitory factor, recurrent pregnancy loss

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Recurrent pregnancy loss (RPL) is a distinct disorder defined by ≥2 failed clinical pregnancies (1). Estimates suggest that within their reproductive age span, less than 5% of women will face two consecutive miscarriages, but about 1% will experience ≥3 consecutive miscarriages (2). The proposed causes of RPL include anatomic, genetic, endocrine, autoimmune, and infectious origins; however, nearly 50% of cases of RPL are idiopathic (2). Inflammatory processes may play a role in idiopathic RPL. How the fetus avoids rejection from the maternal immune system is still an inadequately understood mechanism (3). Despite the apparently immunologically privileged status of the fetus, human implantation requires inflammatory mediators for attachment and implantation.

Implantation is managed by a complex interaction between the endometrium and blastocyst in which several cytokines and adhesion molecules play a vital role during the midsecretory phase of the menstrual cycle. This period is also known as the implantation window (4). During the implantation window, specific expressions of adhesion molecules and cytokines can be observed (5).

Cytokines produced by T cells are important mediators of signals between cells of the immune system and other cells. If a change occurs in the

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production pattern of cytokines by T cells, it may have an effect on immunologic tolerance. There are a few T-helper (T_H) cells. For instance, T_H1 cells produce cytokines such as interferon-γ (IFN-γ) that have a role in cellular immunity, whereas T_H2 cells produce anti-inflammatory cytokines that function in humoral immunity (6). Interleukin-12 (IL-12) induces the differentiation of T_H1 cells through the production of IFN-γ (7, 8). IL-18 then acts as a mediator to increase IFN-γ production (9, 10). Researchers have demonstrated that T_H1-type immunity may have a detrimental role during pregnancy because T_H1-dependent mechanisms are potentially involved in allograft rejection (11, 12). There is also a debate as to the importance of T_H2-type immunity during pregnancy because it maintains allograft tolerance (13–15). In summary, it has been suggested that the T_H2-type cytokine response is vital for a successful pregnancy, whereas an augmented T_H1-type cytokine response may result in pregnancy loss (16). This is also known as the T_H1/T_H2 paradigm. The paradigm postulates that the fetus is not rejected by the maternal immune system because pregnancy is a predominance of anti-inflammatory mediators (T_H2-type immunity), which overrules T_H1-type immunity and subsequently protects the fetus in the maternal uterus (16–20).

Intercellular adhesion molecule-1 (ICAM1) is expressed in the epithelium and endothelium, and is regulated by cytokines including IFN-γ (21–23). Its expression pattern may illustrate the interactions of the various factors involved in the implantation process because ICAM-1 is a cytokine inducible molecule and related to the menstrual cycle (24).

Leukemia inhibitory factor (LIF) has an important role in embryo development and implantation (25). Defective production of LIF by T cells has been reported in patients with RPL (18). Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine and is also involved in the immune response (26). In addition, studies in mice and humans have shown that MIF has a role in various reproductive processes (27–29).

To the best of our knowledge, no studies have examined levels of cytokines produced by T_H1/T_H2 cells, namely, IL-12, IL-18, IFN-γ, LIF, ICAM-1, and MIF, together in precisely timed blood and endometrial tissue samples within the implantation window from women with idiopathic RPL. We hypothesized that the levels of these biomarkers both in blood and endometrial tissue would be different in patients with idiopathic RPL compared with healthy controls. The secondary objective was to evaluate the correlation between blood levels and tissue samples of these selected biomarkers.

MATERIAL AND METHODS

Patients, Blood, and Endometrial Samples

Blood samples and endometrial biopsies were obtained from women who attended the gynecology and infertility clinics of Istanbul University School of Medicine (Istanbul, Turkey). Samples were collected after the patients gave informed consent. Approval from the ethics committee of Istanbul University School of Medicine was obtained for this study.

Idiopathic RPL was determined by exclusion of autoimmune, anatomic, infectious, genetic, endocrine, and infectious factors. Inclusion criteria for the study group were [1] ≥ 2 consecutive failed clinical pregnancies, [2] normal female karyotype, [3] no pathologies detected in pelvic ultrasonography, [4] no pathologies detected in hysterosalpingography, [5] negative screening for antiphospholipid syndrome, [6] no inherited and/or acquired coagulopathies, [7] no chronic disorders, [8] normal day-3 hormone profile, including follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E_2), prolactin, and thyroid-stimulating hormone (TSH), [9] last pregnancy loss at least 6 months ago and no previous live births, and [10] no uterine pathologies detected during office hysteroscopy.

The control group included fertile women who had regular menstrual cycles with a history of at least one live birth, no history of abortion or infertility, and who were admitted for annual gynecologic examination. In both groups, no women had taken steroid hormones or antibiotics that could potentially affect the analyses. Smokers were also excluded in both groups. All participants were required to be aged between 20 and 40 years.

Blood samples were drawn during the midsecretory phase (days 19–23) within the implantation window in the morning. The samples were centrifuged at 3,000 rpm for 10 minutes at 4°C to aliquot the supernatants, which were stored at −80°C until the enzyme-linked immunosorbent assay (ELISA) analysis. Endometrial sampling using a Pipelle was performed on the same day. Tissue samples were stored at −80°C until ELISA analysis. For all women, the serum β-human chorionic gonadotropin (β-hCG) test for pregnancy was negative (<25–35 mIU/mL), and the serum progesterone levels were >10 ng/mL on the day of sampling, which showed that the patients had ovulated.

Determination of IL-12, IL-18, IFN-γ, ICAM-1, LIF, and MIF in Blood and Tissue Using ELISA

Expression levels of IL-12, IL-18, IFN-γ, ICAM-1, LIF, and MIF levels were determined using commercially available ELISA kits (MyBioSource) in accordance with the manufacturer's instructions. For ELISA analyses of blood samples, thawed supernatants were centrifuged at 10,000 rpm for 5 minutes at 4°C to remove any precipitate. For ELISA analyses of tissue samples, homogenization was achieved using an electric homogenizer with the addition of an extraction buffer. Constant agitation was maintained for 2 hours at 4°C. The samples were then centrifuged for 20 minutes at 13 000 rpm at 4°C to aliquot the supernatant. The intra-assay and interassay coefficients of variation for all measurements were <5% and 10%, respectively.

Statistical Analysis

Descriptive data were expressed as mean ± standard deviation (SD). To compare the differences of the studied parameters (IL-12, IL-18, IFN-γ, ICAM-1, LIF, and MIF) between women with RPL and age-matched fertile controls, Student's *t*-test was used. Pearson's correlation was used to analyze the relation between blood and tissue measurements. Binary logistic regression analysis was used to