Macrophage migration inhibitory factor as a potential biomarker of endometriosis

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Objective: To evaluate the expression of *MIF*, *CD74*, and *COX-2* in normal, ectopic, and eutopic endometrium during the menstrual cycle and to assess MIF level in peripheral blood.
Design: The expressions of *MIF*, *CD74*, and *COX-2* in normal, ectopic, and eutopic endometrium were evaluated with the use of real-time polymerase chain reaction. MIF protein in peripheral blood samples was checked with the use of ELISA.
Setting: Reproductive biomedicine research center.
Patient(s): Sixteen normal women and 20 women with endometriosis.
Intervention(s): Ectopic biopsies were obtained with the use of laparoscopic procedure, and eutopic and control biopsies were obtained with the use of Pipelle. Peripheral blood samples were collected before laparoscopy.
Main Outcome Measure(s): The expression of *MIF*, *CD74*, and *COX-2* in normal, ectopic and eutopic endometrium during the menstrual cycle and the expression level of MIF in peripheral blood samples.
Result(s): Relative mRNA expression of *MIF*, *CD74*, and *COX-2* were significantly higher in ectopic endometrium than in eutopic and control endometrium. Also, there were significant differences in expression of these genes in normal, ectopic, and eutopic endometrium during the menstrual cycle. Moreover, women with endometriosis had significantly higher circulating levels of MIF compared with control subjects.
Conclusion(s): Dynamic expression of *MIF*, *CD74*, and *COX-2* during the menstrual cycle could play an essential role in reproduction, inflammation, and endometrium reconstruction. A higher expression of these genes in ectopic endometrium can be considered as a molecular biomarker for endometriosis development and pathophysiology. Also, a high level of MIF in blood serum can act as a biomarker in the diagnosis of endometriosis. (Fertil Steril® 2015;103:153–9. ©2015 by American Society for Reproductive Medicine.)
Key Words: MIF, CD74, COX-2, endometriosis

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Endometriosis is classically defined as the presence of functional endometrial glands and stroma outside the uterine cavity. About 15%–20% of women suffer from this disease in their reproductive life, with symptoms consisting of pelvic pain, dysmenorrhea, dysfunctional uterine bleeding, gastrointestinal, dyspareunia, and infertility (1). The incidence of endometriosis is associated with the selection of diagnostic methods and sampling framework (2).

The etiology of endometriosis is still poorly defined. Endometriosis is a multifactorial disorder, but the absolute causes are unknown although some of the possible reasons include hormonal factors, genetics, environmental factors, and alterations in the endocrine or immune systems (2, 3).

Among the theories proposed to explain the pathogenesis of endometriosis, the Sampson theory is by far the most widely accepted. It proposes that endometrial cells are spread by

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retrograde menstruation through the fallopian tubes into the peritoneal cavity (4). In women who don't have endometriosis, the ectopic endometrial cells are eliminated by immune cells such as macrophages, natural killer cells, and lymphocytes (5, 6). Dysfunction of immune cells and immunologic factors are mechanisms that cause the survival and development of ectopic endometrial cells (7). Immunologic changes that occur in patients with endometriosis include reduced natural killer cell and T-lymphocyte cytotoxicity in the peritoneal fluid and an elevated number of activated macrophages (5, 8). After the implantation of ectopic endometrial cells, secretion of growth and angiogenesis factors by immune cells increases. Therefore, the number of ectopic endometrial cells increases and development of inflammation occurs. There is an increase in macrophages where ectopic endometrial cells have been implanted (7). The macrophages existing in the inflammatory areas secrete macrophage migration inhibitory factor (MIF) (9). MIF is a proinflammatory factor involved in T-cell activation, cell growth, apoptosis inhibition, and increase of angiogenic factors (10, 11). Only a single MIF gene exists in humans, located on chromosome 22 (22q11.2) (12).

MIF, via its receptor molecule, CD74, initiates a signaling cascade that leads to proliferation and survival of cells. This signaling cascade also helps to maintain a larger number of T cells, B cells, and macrophages during inflammation (13). MIF, via binding to CD74, activates the p38 signaling pathway which leads to a positive effect on the expression of *COX-2* (7). Following this background information, we hypothesized that overexpression of *MIF*, *CD74*, and *COX-2* may initiate a signaling cascade that leads to proliferation and survival of endometrial cells. Therefore, the primary objective of this study was to evaluate the expression of *MIF*, *CD74*, and *COX-2* genes in normal, ectopic, eutopic endometrium samples. The level of MIF in peripheral blood samples was another variable that was checked in this study. The secondary objective was to examine the correlation between expression of these genes and different stages of the menstrual cycle in women with and without endometriosis.

MATERIALS AND METHODS

Tissue Collection for Genomic Studies

This study was approved by the Institutional Ethics Committee of Royan Institute, and written informed consents were obtained before the collection of tissue samples. Endometrial biopsy specimens were collected from 20 endometriosis patients. All patients were 20–45 years old, consulting for infertility and/or pelvic pain, and found to have no endometrial hyperplasia or neoplasia. The stage of endometriosis was determined according to the revised classification of the American Fertility Society (14). All patients in this study were in stage III or IV. Sixteen normal endometrium samples during the menstrual cycle were tested as control group in this study. The control group taking part in this investigation were 20–40 years old having regular cycles, showing no evidence of any pathologic uterine disorder, and had not used oral contraception or an intrauterine device in the previous 3 months. None had visible endometrial hyperplasia or neoplasia, inflammatory disease, or endometriosis at the time of clinical examination or laparoscopy. The cycle day was determined according to the cycle history and histologic criteria. Each of the control women had at least one child by natural conception.

Both ectopic and eutopic endometrium samples were investigated in women with endometriosis. Ectopic biopsies were obtained with the use of laparoscopic procedure, and eutopic biopsies were obtained with the use of Pipelle. All of the ectopic endometrial tissues were endometrioma. In women without endometriosis, control biopsies were obtained with the use of Pipelle. Each tissue samples was divided into small (1 × 1 cm) pieces that were immediately placed in RNA later solution (Ambion) and stored at −80°C until processing.

To compare the relative expression of *MIF*, *CD74*, and *COX-2* genes in normal endometrium during the menstrual cycle, we divided the biopsies into the following three groups: menstrual (last menstrual period [LMP] + 1–4 days), proliferative (LMP + 5–14 days), and secretory (LMP + 15–29 days) phases. To compare the relative expression of these genes during the menstrual cycle, ectopic and eutopic endometrium samples were collected from the proliferative and secretory groups (Table 1).

Total RNA Purification and cDNA Synthesis

Total RNA was extracted separately from each group with the use of TRI reagent (Sigma) and treated with DNase I (Fermentas). First-strand cDNA synthesis was performed with the use of random hexamer primers and the superscript II reverse transcriptase system (Fermentas). For ensuring cDNA synthesis, the products were checked with the use of human β-actin as a housekeeping gene (Metabion) and platinum Blue PCR

TABLE 1

Designation of sample groups analyzed in the study.

Tissue and blood samples	No. of samples	Age (y) (mean ± SD)
Endometriosis		
Ectopic and eutopic endometrium		
Proliferative phase	15	29.4 ± 1.3
Secretory phase	5	28.8 ± 2.6
Blood		
Menstrual phase	3	33 ± 1
Proliferative phase	7[a]	28.4 ± 1.46
Secretory phase	5[a]	28.8 ± 2.65
Control		
Endometrium		
Menstrual phase	3	30.3 ± 1.4
Proliferative phase	5	33 ± 1.7
Secretory phase	4	32 ± 1.15
Blood		
Menstrual phase	3[a]	31.33 ± 1.6
Proliferative phase	5[a] + 3	30.8 ± 1.6
Secretory phase	4[a] + 1	31 ± 1.2

[a] Indicates the blood samples for which tissue samples were analyzed in parallel.

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