ORIGINAL ARTICLE: ENDOMETRIOSIS

Pathophysiologic processes have an impact on the plasma metabolomic signature of endometriosis patients

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Objective: To evaluate potential variations in the plasma metabolomic profile of endometriosis patients as a consequence of pathophysiologic alterations associated with this disorder.
Design: Prospective study. For each subject, a plasma sample was collected after overnight fasting and before surgery.
Setting: University medical center.
Patient(s): The clinical cohort included 50 endometriosis patients, diagnosed at early (n = 6) and advanced (n = 44) stages of the disease, and 23 healthy women. All volunteers underwent diagnostic laparoscopy to visually confirm the presence or absence of endometriotic lesions.
Intervention(s): Metabolomic profiling of plasma samples based on ^{1}H-nuclear magnetic resonance (NMR) spectroscopy in combination with statistical approaches.
Main Outcome Measure(s): Comparative identification of metabolites present in plasma from endometriosis patients and healthy women.
Result(s): The plasma metabolomic profile of endometriosis patients was characterized by increased concentration of valine, fucose, choline-containing metabolites, lysine/arginine, and lipoproteins and decreased concentration of creatinine compared with healthy women. Metabolic alterations identified in the plasma metabolomic profile of endometriosis patients correlate with pathophysiologic events previously described in the progression of this disease.
Conclusion(s): The results highlight the potential of ^{1}H-NMR–based metabolomics to characterize metabolic alterations associated with endometriosis in plasma samples. This information could be useful to get a better understanding of the molecular mechanisms involved in the pathogenesis of endometriosis, thus facilitating the noninvasive diagnosis of this pathology at early stages. (Fertil Steril® 2016;■: ■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: Endometriosis, metabolomics, ^{1}H-NMR spectroscopy, plasma, biomarkers

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Endometriosis is one of the most common gynecologic disorders in women of reproductive age. It is an estrogen-dependent chronic condition defined by the presence of endometrial glands and stroma in ectopic locations (1). Clinical manifestations of endometriosis can vary from asymptomatic and unexplained infertility to severe dysmenorrhoea, dyspareunia, chronic pelvic pain, and fatigue (2, 3). This condition has an important socioeconomic impact, because it decreases significantly the quality of life of the patients and is responsible for substantial health expenditures regarding both diagnosis and treatment (4).

Currently, the criterion standard to diagnose and stage endometriosis is based on direct visualization of the lesions, usually through laparoscopy, and subsequent histologic verification of endometrial glands and/or stroma (3). However, the enormous variability and nonspecificity of the symptoms, its clinical parallelism with other disorders, and, partly, the requirement for surgical diagnosis (1, 5) contribute to the underdiagnosis of endometriosis and translate to an average of 8 years of latency from onset of symptoms to definitive diagnosis (6). In this

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context, the development of reliable noninvasive diagnostic tests remains one of the top priorities in the endometriosis field (7). It would reduce the multiple drawbacks associated with the inherent risks of surgical procedures (8), increasing the accessibility to diagnostic procedures, and improving treatment outcomes.

"Omics"-based studies provide a powerful tool for getting a deeper insight into the nature of complex disorders such as endometriosis. Among them, metabolomics studies based on the analysis of different biologic matrices are progressively being adopted for measuring system-wide alterations of metabolic pathways associated with phenotypic perturbations (9). In particular, metabolomics with the use of nuclear magnetic resonance (NMR) spectroscopy represents an attractive choice for clinical studies owing to its quantitative and nondestructive nature (10). Recent studies (11–13) show the potential of this technology to unravel molecular mechanisms involved in different gynecologic conditions, information that could be useful in the prediction, diagnosis, and monitoring of these pathologic processes (14).

A previous study carried out by our group based on the analysis of the urine metabolomic profile of endometriosis patients (15) revealed that metabolites involved in inflammation and oxidative stress play a very important role in endometriosis. Building on those results, the aim of the present study was to contribute, based on the characterization of the plasma metabolomic profile of endometriosis patients, to the identification of metabolic alterations associated with this disorder. The results of this study revealed additional metabolic changes associated with cell proliferation, glycome alteration, inflammation, and oxidative stress. Taken together, the analysis of both the urine and the plasma metabolomic profiles of endometriosis patients provides relevant information for getting a better understanding of the pathophysiologic mechanisms of endometriosis and could contribute to the noninvasive identification of relevant biomarkers of this disease.

MATERIALS AND METHODS

Study Design and Subjects

Patient recruitment was carried out at the Hospital Universitario La Fe (Valencia, Spain), and measurements and analysis of the plasma metabolomic profiles were performed at the Centro de Investigación Príncipe Felipe (Valencia, Spain). Peripheral blood samples were collected from 73 volunteers (18–40 years old, body mass index ≤ 25 kg/m^2) recruited consecutively from November 2009 to December 2012. Clinical diagnosis and classification of subjects were performed through laparoscopy to visually confirm the presence or absence of endometriosis. The group of women diagnosed with endometriosis consisted of 50 patients presenting endometriosis-associated symptoms (dysmenorrhea, dyspareunia, deep pelvic pain, infertility, etc.) with vaginal ultrasound scans showing endometriomas and/or rectovaginal nodules who were scheduled for laparoscopy for diagnosis and surgical treatment. Endometriosis patients were staged from I to IV according to the classification system of the revised American Society for Reproductive Medicine score (16). Six plasma samples were collected from women at early stages (minimal/mild) and 44 from women at advanced stages (moderate/severe). The control group was composed of 23 healthy women who underwent routine diagnostic laparoscopy for tubal sterilization and, after discarding the presence of endometriosis, were included in the healthy individuals cohort. Menstrual cycle phases (MCPs) were classified as follicular phase (cycle days 1–14) or luteal phase (cycle days 15–28) after adjustment to a 28-day cycle and confirmation of the endometrial pattern with the use of vaginal ultrasound. Women with previous history of endometriosis or other gynecologic diseases, including fibroids, other ovarian cysts, and pelvic inflammatory disease, were excluded from the study. None of the participating women had received hormonal therapy for ≥ 1 month before surgery or preoperative medication before sample collection. In addition, women diagnosed with any other disease or under pharmacologic treatment were not included in the study. Despite the difficulty of recruiting endometriosis patients and healthy women fulfilling the inclusion criteria defined in this study, the sample size exceeded the estimation provided with the use of Metsizer (17), a method used in metabolomic studies for determining sample size that does not require previous pilot data or assumed variable independence. Clinical information associated with each sample group is summarized in Table 1.

Patient recruitment and sampling procedures were performed in accordance with the Declaration of Helsinki and applicable local regulatory requirements and laws and after approval from the ethics committee of the Hospital Universitario La Fe (Valencia, Spain). Written informed consent was obtained from each participant before being included in this study.

Sample Collection and NMR Sample Preparation

Blood samples were collected from each participant after overnight fasting and before surgery and anesthesia induction into Li-heparin tubes to complete the vacuum (BD vacutainer, Li-heparin). After homogenization, samples were centrifuged at 1,600*g* for 15 minutes at 4°C. The supernate

TABLE 1

Clinical characteristics of the samples included in the plasma metabolomics study.

Characteristic	Healthy women (n = 23)	Endometriosis patients (n = 50)
Age (y), mean ± SD	34.30 ± 5.04	31.06 ± 5.52
Menstrual cycle phase, n		
Follicular phase	22	39
Luteal phase	1	11
Body mass index (kg/m^2)		
Mean ± SD	21.99 ± 1.69	21.15 ± 1.55
Underweight (<18.50), n	–	1
Normal weight (18.50–25.00), n	23	49
Overweight (>25.00), n	–	–
Obese (>30.00), n	–	–
Stage of the disease		
Minimal/mild (I/II)	–	6
Moderate/severe (III/IV)	–	44

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