Role of 8-iso-prostaglandin $F_{2\alpha}$ and 25-hydroxycholesterol in the pathophysiology of endometriosis

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Objective: To investigate the involvement of 8-iso-$PGF_{2\alpha}$ and 25-hydroxycholesterol (25-OH-Chol) in the pathophysiology of endometriosis.
Design: Observational case-control study using enzyme immunoassay and high-performance liquid chromatography (HPLC).
Setting: Postgraduate Institute of Medical Education and Research.
Patient(s): Forty-five women undergoing laparoscopy (n = 25), laparotomy (n = 19), or tubal ligation (n =1).
Intervention(s): Venipuncture and laparoscopic peritoneal fluid (PF) collection.
Main Outcome Measure(s): The levels of 8-iso-$PGF_{2\alpha}$ were determined both in urine and PF of all the patients using enzyme immunoassay. The levels of 25-OH-Chol were determined by using reversed phase HPLC both in the plasma and PF samples. Oxidative damage to DNA was assessed by agarose gel electrophoresis.
Result(s): Significantly increased levels of 8-iso-$PGF_{2\alpha}$ were observed both in urine and PF of women with endometriosis compared with control women. Similarly, higher levels of 25-OH-Chol were observed both in plasma and PF of patients compared with controls and the difference was statistically significant. A clear-cut tailing pattern was observed in DNA of patients with endometriosis, indicating significant DNA damage.
Conclusion(s): Our observations implicate oxidative stress in the pathophysiology of endometriosis. For the first time, we demonstrate that 8-iso-$PGF_{2\alpha}$ and oxysterols (the known promoters of steroidogenesis) might be the culprits in this disease. (Fertil Steril® 2010;94:63–70. ©2010 by American Society for Reproductive Medicine.)

Key Words: Endometriosis, oxidative stress, 8-iso-$PGF_{2\alpha}$, 25-hydroxycholesterol, oxidative DNA damage

Endometriosis, characterized by the ectopic presence of endometrial glandular and stromal cells, is a common benign gynecological disease with poorly understood pathogenesis. The estimated prevalence of endometriosis in asymptomatic women is estimated in the range of 2%–50%, depending on the diagnostic criteria (1). An immunologic/inflammatory etiology has been postulated in endometriosis, as demonstrated by increased concentrations of activated macrophages, cytokines, T cells, and B cells in the pelvic cavity.

It still remains an open question as to what extent the peritoneal environment influences the establishment or progression of endometriosis. In women with endometriosis, scientific evidence dictates that peritoneal fluid (PF) has increased levels of tumor necrosis factor-α (TNF-α), interleukin-1, antibodies, and reactive oxygen species (ROS). It is rich in lipoproteins, particularly low-density lipoprotein (LDL), which generates oxidized lipid components in a macrophage-rich inflammatory milieu (2). Therefore, establishment of a chronic inflammatory response, together with the presence of a local oxidative environment, could play an important role in the etiology and progression of endometriosis.

The oxidants exacerbate the progression of endometriosis by inducing chemoattractants such as monocyte chemotactic protein-1 (MCP-1) and endometrial cell growth-promoting activity (2–4). An obvious mechanism, through which oxidative stress might impair vital functions, is oxidative damage to critical biomolecules including proteins, lipids, and DNA (5–8). Activated macrophages in the peritoneal cavity generate oxidative stress with resultant production of lipid peroxides, their degradation products, and products formed from their interaction with LDL, apolipoproteins, and other proteins (4).

The F2-isoprostanes are a complex family of compounds generated by nonenzymatic peroxidation of arachidonic acid (9) on cell membranes (10) and LDL particles (11). Various studies have documented increased 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) as a reliable, sensitive and a specific biomarker of lipid peroxidation in vivo (7, 12, 13), whereas other products of the isoprostane pathway, such as D2- and E2-isoprostanes, are less suitable because of their lesser stability. The measurement of F2-isoprostanes is the most reliable approach to assess oxidative stress status in vivo,

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and the products of the isoprostane pathway have been found to exert potent biological actions, therefore could act as pathophysiological mediators of the disease (7). The 8-iso-$PGF_{2\alpha}$ not only acts as a vasoconstrictor, but has also been shown to promote mitogenesis and cell adhesion of monocytes and polymorphonuclear cells to endothelial cells and promotes induction of endothelial cell necrosis (14). Hence, measurement of F2-isoprostanes may have prognostic value in those diseases in which a role for oxidative stress has been implicated (7) and thus, could be a major link in the infertility puzzle, as well as in some reproductive organ diseases such as endometriosis (15).

Furthermore, oxidative modification of LDL is associated with oxidation of cholesterol to yield oxygenated species such as oxysterols and hydroxyl-fatty acids, which can be used as oxidative stress markers for determining in vivo lipoprotein oxidation (16). Oxysterols have various biological activities, are cytotoxic, have been shown to promote tissue inflammation and necrosis, produce immunosuppression, enhance steroid biosynthesis, and act as ligands for certain nuclear receptors, through which they regulate cholesterol homeostasis (17). Among various oxysterols, 25-hydroxycholesterol (25-OH-Chol) is the most potent suppressor of hydroxyl-methylglutaryl coenzyme A reductase (18).

Oxysterols have a tendency to bind to intracellular antiestrogen-binding sites and thus, influence the estrogen (E) level (16). Evidence in the literature dictates that E is the best-defined mitogen for growth and inflammatory processes in the ectopic endometriotic tissue (19). Hence, there is a possibility that 25-OH-Chol may be responsible for increased levels of E locally in the endometriotic disease. Therefore, it seems prudent to explore the role that oxysterols play in endometriosis, a condition with high prevailing E.

Because conditions of oxidative stress are invariably associated with inflammation in inflammatory disorders like endometriosis (3, 4, 15, 20), identification of newer biomarkers can help to define the disease pattern. We, therefore, hypothesized that oxidative stress might be prevalent in patients with endometriosis and could influence establishment or progression of the disease. Therefore, in the present study we have tried to investigate the in vivo levels of 8-iso-$PGF_{2\alpha}$ and 25-OH-Chol, both in the PF and plasma, along with oxidative damage to DNA as possible biomarkers that may help predict the course of the disease.

MATERIALS AND METHODS

The patients (n = 45) enrolled in the present study were women undergoing diagnostic laparoscopy for evaluation of pelvic pain/endometriosis (n = 25) or laparotomy for endometriotic cystectomy and adhesionolysis (n = 19) or laparoscopic tubal ligation (n = 1) at the Postgraduate Institute of Medical Education and Research, Chandigarh, India.

All the patients included in the study were of reproductive age and in the early proliferative cycle phase at the time of sampling. For each patient enrolled in the study, a questionnaire was filled out where clinical history as well as complete information regarding personal habits such as smoking and alcohol intake, menstrual characteristics, crude evaluation of the intensity of pain, and contraception and parity was recorded. The patients were also asked about their intake of drug/medication, if any (e.g., danazol, antihypertensives, antioxidants [vitamins C and E]). However, none of them were smokers or alcohol users and were not on any antihypertensive or antioxidant medications (Table 1). Thirty women with laparoscopically proven endometriosis were included in group I (Table 1). Patients with genital cancer, pregnancy, active pelvic inflammatory disease, and presence of any other acute or chronic disease, such as diabetes, tuberculosis, and cardiovascular disease, were excluded from the study. Endometriosis was diagnosed using the revised classification of the American Fertility Society (AFS) based on the location, bulk of the disease, and amount and severity of adhesions (21) and was confirmed histologically (Table 1). The patients in group I were further divided into two groups depending on whether they were (group Ia) (n = 11) or were not on danazol (group Ib) (n = 19).

Fifteen women without endometriosis, who had a normal pelvic anatomy and were undergoing tubal ligation (n = 1) or laparoscopy for unexplained infertility (n = 14), were included as controls in group II (Table 1). All of the control patients were free of adhesions and endometriosis, genital cancer, pregnancy, active pelvic inflammatory disease, and presence of any other acute or chronic disease such as diabetes, tuberculosis, and cardiovascular disease.

A fully informed written consent was taken from all the patients before their participation in the study. The study was approved by the Institutional Ethics Committee of Postgraduate Institute of Medical Education and Research, Chandigarh, India.

Biochemical laboratory investigations included analysis of 8-iso-$PGF_{2\alpha}$ levels in the samples of urine and PF, 25-OH-Chol in plasma and PF, and oxidative damage to DNA, isolated from the whole blood of all the study subjects. All the chemicals, reagents, and standards were of molecular biology grade and were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). The chemicals and reagents required for high-performance liquid chromatography (HPLC) were purchased from Merck Diagnostics (Darmstadt, Germany) and were of HPLC grade.

Preparation of Urine, Peritoneal Fluid, and Plasma Samples

Spot urine samples were obtained from all participants before the surgical procedure in sterile containers containing butylated hydroxytoluene as an antioxidant. The urine samples were centrifuged at 1,500–2,000 rpm for 10 minutes to remove any sediment. The supernatants were collected and stored at -80°C until further analysis of 8-iso-$PGF_{2\alpha}$.

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