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

Indu Sharma, M.Sc.,a Lakhbir Kaur Dhaliwal, M.D.,b Subhash Chand Saha, M.D.,b
Sonal Sangwan, M.Sc.a, and Veena Dhawan, Ph.D.a

a Department of Experimental Medicine and Biotechnology and b Department of Obstetrics and Gynecology, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India

Objective: To investigate the involvement of 8-iso-PGF\(F_{2\alpha}\) 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\(F_{2\alpha}\) 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\(F_{2\alpha}\) 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\(F_{2\alpha}\) 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\(F_{2\alpha}\), 25-hydroxycholesterol, oxidative DNA damage

Received September 17, 2008; revised January 9, 2009; accepted January 26, 2009; published online March 26, 2009.

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-\(\alpha\) (TNF-\(\alpha\)), 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\(F_{2\alpha}\)) 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,
and the products of the isoprostane pathway have been found to exert potent biological actions, therefore could act as pathophysiologically 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-isoprostanates 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 antioestrogen-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 adhesiolysis ($n = 19$) 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$_{2\alpha}$. 

Sharma et al. 8-iso-PGF$_{2\alpha}$ and 25-OH-Chol in endometriosis Vol. 94, No. 1, June 2010
Five milliliters of venous blood samples were collected from the antecubital vein in ethylenediaminetetraacetic acid (EDTA) vacutainers from the overnight fasted patients before anesthesia. The blood samples were used for genomic DNA isolation and plasma separated and stored at -80°C for subsequent determination of 25-OH-Chol.

The PF samples were collected from the pouch of Douglas into a sterile syringe at the time of the diagnostic surgical procedure from all the patients. Each sample was transferred to a heparinized container and centrifuged at 1,500–2,000 rpm for 10 minutes. The supernatant was collected and stored at -80°C until further analysis of 8-iso-PGF$_{2\alpha}$ and 25-OH-Chol.

### 8-iso-PGF$_{2\alpha}$ Assay

The level of 8-iso-PGF$_{2\alpha}$ was determined in urine and PF of patients and controls using a commercially available enzyme immunoassay kit (900-010; Assay Designs, Inc., Minneapolis, MN) according to the manufacturer's instructions. Data for 8-iso-PGF$_{2\alpha}$ is expressed in nanograms per milliliter. The detection limit of quantification was 5 pg/mL. The intra-assay and interassay coefficients of variation (CV) were 5.0% and 5.5%, respectively.

### 25-OH-Chol Assay

The level of 25-OH-Chol was determined in the plasma and PF samples by the method of Burkard et al. (22) with some

---

**TABLE 1**

Baseline characteristics of all the patients.

<table>
<thead>
<tr>
<th>Clinical feature</th>
<th>Endometriosis (n = 30)</th>
<th>Controls (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (y)</strong></td>
<td>24–42</td>
<td>25–39</td>
</tr>
<tr>
<td>Range</td>
<td>31.03 ± 1.05</td>
<td>31.06 ± 1.56</td>
</tr>
<tr>
<td>Mean</td>
<td>24.97 ± 0.47</td>
<td>24.3 ± 0.57</td>
</tr>
<tr>
<td><strong>BMI (kg/m$^2$)</strong></td>
<td>120 ± 1.5</td>
<td>121 ± 2.5</td>
</tr>
<tr>
<td><strong>Blood pressure (mm Hg)</strong></td>
<td>80 ± 0.53</td>
<td>79 ± 1.37</td>
</tr>
<tr>
<td><strong>Systolic blood pressure</strong></td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td><strong>Laparotomy</strong></td>
<td>19</td>
<td>Nil</td>
</tr>
<tr>
<td><strong>Laparoscopic tubal ligation</strong></td>
<td>Nil</td>
<td>1</td>
</tr>
<tr>
<td><strong>Smoking</strong></td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td><strong>Alcohol intake</strong></td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td><strong>Danazol treatment</strong></td>
<td>11</td>
<td>Nil</td>
</tr>
<tr>
<td><strong>Type of infertility</strong></td>
<td>22</td>
<td>10</td>
</tr>
<tr>
<td><strong>Primary</strong></td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td><strong>Secondary</strong></td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td><strong>No infertility problem</strong></td>
<td>1</td>
<td>Nil</td>
</tr>
<tr>
<td><strong>Duration of infertility (y)</strong></td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>&lt;2</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>2–4</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td><strong>Severity of disease (revised AFS classification)</strong></td>
<td>1</td>
<td>Nil</td>
</tr>
<tr>
<td><strong>Stage I</strong></td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td><strong>Stage II</strong></td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td><strong>Stage III</strong></td>
<td>23</td>
<td></td>
</tr>
<tr>
<td><strong>Stage IV</strong></td>
<td>1</td>
<td>Nil</td>
</tr>
<tr>
<td><strong>Dysmenorrhea</strong></td>
<td>19</td>
<td>3</td>
</tr>
<tr>
<td><strong>Yes</strong></td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td><strong>No</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:** Results are expressed as mean ± SEM.

minor modifications. Briefly, plasma and PF samples were saponified, followed by centrifugation at 2,500 rpm for 10 minutes. The supernatants containing oxysterols were cleaned up by solid-phase extraction through preconditioned C18 cartridges (Sep-Pak C18 OH; Waters; Millipore Corp, Milford, MA). A mixture of n-heptane and 2-propanol (50:50; vol/vol) was used as the mobile phase and the samples were eluted. Eluted samples were then evaporated at 30°C and the residues were dissolved in 100 μL of methanol, which was then injected into the HPLC system. The HPLC instrumentation used was from Waters India Ltd., (pump controller, model No. 600; autosampler, model No. 717; and photodiode array detector, model No. 996; Bangalore, India). The column used was a Bondapak C18 reversed phase column (3.9 mm × 30 cm). Acetonitrile/water was used as the mobile phase and the oxysterol peaks were detected at λ = 210 nm. The unknown samples were validated against standard 25-OH-Chol, which was procured from Sigma-Aldrich Chemicals Co.

DNA isolation and Gel Electrophoresis
Genomic DNA was extracted from whole blood of all the study subjects by using the method of Lahiri et al. (23). DNA damage was determined by loading the samples on 0.8% agarose gel, as previously described (6).

Statistical Analysis
All results are represented as mean ± standard error of the mean (SEM). Comparison of the variables between patient and control groups was performed using unpaired Student’s t-test. Pearson’s coefficient of correlation (r) was also determined for levels of 8-iso-PGF2α and 25-OH-Chol. All analyses were performed using the statistical software, SPSS 10.0 (SPSS Inc., Chicago, IL).

RESULTS

8-iso-PGF2α
The levels of 8-iso-PGF2α were determined in urine and PF of the study patients. The urinary levels of 8-iso-PGF2α were found to be significantly higher in patients with endometriosis compared with control patients (P<.001) (Table 2). Similarly, we also observed significantly higher levels of 8-iso-PGF2α in the PF of patients with endometriosis when compared with controls (P<.01) (Table 2). When the data were analyzed on the basis of danazol treatment, 8-iso-PGF2α levels in the urine and PF were found to be increased in danazol-treated subjects compared with those subjects who did not receive danazol treatment, but the results were statistically not significant (P>.05) (Table 2).

25-OH-Chol
The levels of 25-OH-Chol were determined in the plasma and PF group I and group II subjects using HPLC. The representative photographs of HPLC profiles of standard 25-OH-Chol, plasma, and PF samples are shown in Figure 1. The levels of 25-OH-Chol levels were observed to be significantly higher in plasma of patients with endometriosis compared with control patients (P<.001) (Table 2). Similarly, increased levels of 25-OH-Chol were observed in the PF of patients with endometriosis compared with control patients (Table 2), and the values were also statistically significant (P<.01). Contrary to our observations with 8-iso-PGF2α,

---

**Table 2**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Endometriosis</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group I (n = 30)</td>
<td>Group Ia (n = 11)</td>
</tr>
<tr>
<td>8-iso-PGF2α (ng/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>226.3 ± 27d</td>
<td>265 ± 28.2e,c</td>
</tr>
<tr>
<td>PF</td>
<td>41.3 ± 3.7d</td>
<td>45.5 ± 6.1a,b</td>
</tr>
<tr>
<td>25-OH-Chol (μg/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>67.1 ± 17.8d</td>
<td>33.5 ± 5.1</td>
</tr>
<tr>
<td>PF</td>
<td>164.8 ± 44.1d</td>
<td>157.2 ± 79.8</td>
</tr>
</tbody>
</table>

*Note: Results are expressed as mean ± SEM; PF = peritoneal fluid.*

a P<.05.
b P<.01.
c P<.001.
d group I vs. group II.
e group Ia vs. group II.
f group Ib vs. group II.

both plasma and PF levels of 25-OH-Chol were found to be higher in patients without danazol treatment, but the difference between danazol-treated and danazol naive patients remained statistically insignificant ($P > .05$) (Table 2).

**Oxidative DNA Damage**

Figure 2 shows the agarose gel electrophoresis patterns of the DNA samples from women with and without endometriosis. A clear-cut significant tailing pattern was observed in samples from Group I subjects indicating significant DNA damage (Fig. 2, lanes 1–4). However, in the case of control subjects, intact bands were observed, indicating no damage (lanes 5–8).

**Correlation Analysis**

The levels of 8-iso-PGF$_{2a}$ (urine and PF) and 25-OH-Chol (plasma and PF) of subjects with endometriosis were analyzed for Pearson’s CV. Those results are shown in Table 3. We observed a significant and positive correlation between urine and PF levels of 8-iso-PGF$_{2a}$ ($r = 0.799; P < .01$). Furthermore, the PF levels of 8-iso-PGF$_{2a}$ were also found to be positively and significantly correlated with PF levels of 25-OH-Chol ($r = 0.385; P < .05$).

**DISCUSSION**

In the present study we investigated the role of 8-iso-PGF$_{2a}$ in urine and PF and 25-OH-Chol in plasma and PF in patients with endometriosis. The results showed that in patients with endometriosis both parameters studied revealed significantly higher levels compared with their control counterparts.

Recent reports in the literature suggest that oxidative stress plays an important role in the normal functioning of the female reproductive system and in the pathogenesis of female infertility (15). Augmented ROS production has the potential to cause cellular damage and dysfunction and various studies implicate oxidative stress as a cause or consequence, inevitably linked to inflammation (22, 24–26). Therefore, we hypothesized that the development of oxidative stress in the local...
peritoneal environment may be one of the links in the chain of events associated with the pathology of endometriosis and may modulate the severity and the dynamics of endometriosis (20, 24). The purpose of the present study was to investigate whether 8-iso-PGF$_{2\alpha}$ and oxysterols, generated under conditions of oxidative stress and inflammation, could serve as reliable biomarkers in discriminating women with endometriosis from those with unexplained infertility and whether these could aid in monitoring the progression of the disease. In support of our hypothesis, we observed significantly increased levels of 8-iso-PGF$_{2\alpha}$ both in urine ($P<.001$) and PF ($P<.01$) of patients with endometriosis compared with their control counterparts. In addition, we observed a positive correlation between levels of 8-iso-PGF$_{2\alpha}$ in urine and PF ($r = 0.799$; $P<.01$) in subjects with endometriosis (Table 3).

Earlier in their study, Sugino et al. (27) had documented that the levels of PGF$_{2\alpha}$ tend to increase toward the late secretory phase of the menstrual cycle and ROS triggered the release of PGF$_{2\alpha}$ from endometriotic stromal cells in vitro. However, in the present study, our data show that the levels of 8-iso-PGF$_{2\alpha}$ were augmented both in the urine and PF samples in those patients who were in the early proliferative phase.

In addition to our observations on 8-iso-PGF$_{2\alpha}$, we also observed significantly higher levels of 25-OH-Chol in both plasma ($P<.05$) and PF ($P<.01$) of group I patients when the data were compared with control patients of group II. Furthermore, in patients with endometriosis, a significant and positive correlation ($r = 0.385$; $P<.05$) was observed between the PF levels of 8-iso-PGF$_{2\alpha}$ and 25-OH-Chol.

Retrograde menstruation is likely to carry highly pro-oxidant factors, such as heme and iron, into the peritoneal cavity, as well as apoptotic endometrial cells, which are well-known inducers of oxidative stress (26). The ROS can alter normal lipid and protein structures of the cells, and can directly cause oxidation of the membrane and cytoplasmic lipids. Furthermore, ROS can directly activate key transcription factors, such as nuclear factor (NF-kB) activation protein-1 (AP-1) and various protein kinases, all of which regulate genes responsible for cell survival, motility, metabolism, and inflammation (11). All of those mechanisms support our observations of finding increased 8-iso-PGF$_{2\alpha}$ and 25-OH-Chol levels in these patients.

The data obtained from this study reconfirm our hypothesis and point in the direction that oxysterols could be one of the important factors responsible for high levels of locally prevailing E in endometriosis. Oxysterols, because of their tendency to bind to intracellular antiestrogen-binding species, could influence the E levels (17). As per literature, E is the best-defined mitogen for the growth of ectopic endometriotic tissue, ensuing inflammation in endometriosis (19). Considerable evidence exists in the literature whereby oxysterols 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, 18). Thus, in view of the data obtained, oxysterols along with 8-iso-PGF$_{2\alpha}$ seem to play a pivotal role in the established endometriotic disease. Ours is the first report that recognizes the presence of these molecules in a disease such as endometriosis.

Furthermore, findings of our study also demonstrated a significant damage to DNA in patients with endometriosis compared with the control group. However, no striking difference in DNA damage was observed between danazol-treated and untreated subjects. Our previous studies have shown that increased oxidative stress and lipid peroxidation lead to oxidative damage to DNA in hypertensive patients (6, 8).
Significantly higher amounts of oxidative damage were detected in the endometrial lesions when compared with normal endometrium, including DNA damage and lipoperoxide contents (15, 28). Thus, our observations on finding augmented 8-iso-PGF$_{2\alpha}$ and 25-OH-Chol levels along with DNA damage in normotensive patients of endometriosis strongly reinforce the fact that indeed, conditions of oxidative stress are prevalent in such patients.

Categorization of the patients on the basis of type of infertility (i.e., primary, secondary, or no infertility), duration of infertility, and grading of the endometriotic cysts (i.e., stage I, II, III, or IV) (Table 1), showed no statistically significant difference in any of the parameters studied (i.e., 8-iso-PGF$_{2\alpha}$ and 25-OH-Chol) when the two groups of patients were compared. One of the reasons could be an uneven distribution of subjects among the different subgroups and also the small number of patients in particular subgroups. Most of the patients under consideration in this study belonged to stage IV of endometriotic disease. However, when these parameters were analyzed in the patients on the basis of danazol treatment, it was notable that 8-iso-PGF$_{2\alpha}$ levels were elevated in patients who were on danazol treatment, whereas oxysterol levels were comparatively attenuated in these subjects. The possible reason behind this finding could be the decreased production of E due to danazol treatment in such patients (29).

Danazol, a derivative of the synthetic steroid ethisterone, is used in the treatment of endometriosis. It lowers E levels by increasing androgen levels and puts the body in a state analogous to menopause. It renders the endometriotic tissue inactive, does not cure it completely, and the condition can revert back within 6 months of stopping the treatment. Danazol has also been shown to increase the LDL and decrease the high-density lipoproteins (HDL) by approximately 40% and 50%, respectively (30).

Furthermore, Crook et al. (31) also observed significantly higher levels of serum lipoprotein(a) in women with endometriosis. Hence, women with endometriosis on danazol treatment are at a higher risk of developing cardiovascular diseases in later stages of their life. Although the exact effects of danazol remain to be elucidated, a cardiovascular risk factor of oxidative stress is added to its properties, as evidenced by the presence of high 8-iso-PGF$_{2\alpha}$ levels in these patients.

The present study suggests that isoprostanes and possibly oxysterols may have a role in the pathogenesis of endometriosis. The observations with limited number of patients in various categories reflect an augmented oxidative milieu with a trend toward higher levels in advanced stages of the disease. This is at best a hypothesis, which must be subjected to further scrutiny.

Our observations provide reasonable grounds that set the stage to test this hypothesis, whether one or all of the parameters tested may serve as biomarkers in endometriosis. However, this needs to be determined further in a larger setup and in other inflammatory diseases of the female genital tract.

Acknowledgments: The authors thank the Gynecology staff of PGIMER, Chandigarh, for their cooperation and help in the sample collection.

REFERENCES


