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European Journal of Obstetrics & Gynecology and Reproductive Biology 123 (2005) 198–203
EUROPEAN JOURNAL OF OBSTETRICS & GYNECOLOGY AND REPRODUCTIVE BIOLOGY
www.elsevier.com/locate/ejogrb

# Concentrations of interleukin (IL)-1α, IL-1 soluble receptor type II (IL-1 sRII) and IL-1 receptor antagonist (IL-1 Ra) in the peritoneal fluid and serum of infertile women with endometriosis☆

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Received 22 July 2004; received in revised form 9 January 2005; accepted 18 April 2005

**Abstract**

*Objective:* Endometriosis is an immune system-related gynaecological disease, characterised by an increase in number and activation of peritoneal macrophages. One of macrophage-derived factors is interleukin (IL)-1. The effects of IL-1 are inhibited by IL-1 receptor type II (IL-1 RII), soluble forms of IL-1 RII (IL-1 sRII) and IL-1 receptor antagonist (IL-1 Ra). The aim of our work was to study the IL-1α, IL-1 sRII and IL-1 Ra levels in the peritoneal fluid (PF) and serum of women with endometriosis in relation to stage of disease.
*Study design:* Concentrations of IL-1α, IL-1 sRII and IL-1 Ra were measured by ELISA assay in the PF and serum of 58 women; 43 with and 15 without endometriosis (control group).
*Results:* Elevated PF and serum IL-1α and IL-1 Ra levels in the women with endometriosis in comparison with the control group were observed. IL-1 sRII levels in PF and serum were higher in the controls than in the women with endometriosis. Concentrations of IL-1α and IL-1 sRII were higher in advanced endometriosis, but higher IL-1 Ra was observed in the early stage of the disease.
*Conclusion:* Impairment of regulation IL-1 activity in the peritoneal fluid and serum of women with endometriosis may play an important role in the pathogenesis and development of the disease.


*Keywords:* Endometriosis; IL-1 family; Peritoneal fluid; Serum

## 1. Introduction

Endometriosis is a common gynaecological disorder of unknown aetiology and pathogenesis. It is well known that endometriosis is often associated with infertility, dysmenorrhoea, pelvic pain and that it affects 5–10% of the female population of reproductive age [1]. Endometriosis is defined as the presence of responsive endometrial glands and stroma tissues that microscopically look like endometrium, outside the uterine, primarily in the peritoneal cavity.

The peritoneal fluid (PF) of women with endometriosis establishes a microenvironment for the development of the disease and undergoes a number of pathological changes, including inflammatory processes with locally and systemically altered function of the immune system [2–4]. Macrophages are the predominant type of cells in PF, which plays a crucial role in the maintenance of humoral and cell-mediated immunity [5,6]. Activated peritoneal macrophages secrete a variety of local products, such as cytokines and growth factors, which can be important in the pathogenesis of endometriosis. Studies indicated that in women with endometriosis levels of macrophage-derived factors such as interleukin (IL)-1, IL-6, IL-10, tumour necrosis factor-alpha (TNF-α), monocyte chemotactic protein-1 (MCP-1) and vascular endothelial growth factor (VEGF) are elevated [7–10].

IL-1, mainly produced by monocytes and macrophages, is an important mediator that plays a key role in immune and inflammatory response in humans. The IL-1 family consists of two distinct molecular forms, IL-1 alpha (IL-1α) and IL-1 beta (IL-1β), which are encoded by different genes, but have comparable biological activities [11]. Cell activations in

☆ The impairment of the mechanisms involved in the regulation of IL-1 actions may be an important factor in the pathogenesis of endometriosis.

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0301-2115/$ – see front matter 
doi:10.1016/j.ejogrb.2005.04.019

response to IL-1 are mediated via the IL-1 receptor type I (IL-1 RI), the functional signalling receptor [12]. However, IL-1 receptor type II (IL-1 RII) is not a signalling molecule and is reported to be a ''decoy'' target of IL-1. Moreover, IL-1 RII could be shed from the cell surface as a soluble molecule (IL-1 sRII) that captures IL-1 and inhibits IL-1 from binding to IL-1 RI [13,14]. A specific inhibitor of IL-1 is also the IL-1 receptor antagonist (IL-1 Ra), which competes with IL-1 for binding to IL-1 RI, without triggering signal transductions and cell activations [15]. It suggests the important role of IL-1 RII, its soluble form IL-1 sRII and IL-1 Ra in regulating the biological activities of IL-1 in the inflammatory sites.

Recently, studies showed the important role of the IL-1 family in endometrium-related disorders, among other things in endometriosis [2–4,9,16]. Data also prominently show a defect in the mechanisms involved in the control of local and systemic IL-1 actions, which may enhance susceptibility for the implantation and growth of endometrial tissue and the consequent development of the endometriosis [16]. Both the defective expression of the cell receptors and most probably the PF environment may be cause of impairment of IL-1 actions.

Based on these data, in the present study, we investigated the levels of IL-1 and the two soluble activity blockers, IL-1 sRII and IL-1 Ra, in the PF and serum of women with endometriosis in relation to the stage of the disease. For this purpose we have measured the concentrations of those cytokines in the PF and serum of women with and without endometriosis.

## 2. Patients and methods

Sixty-nine women between 22 and 38 years of age (mean age: 27.8 ± 3.7 years) undergoing laparoscopy for infertility were included in this study. All women were patients of IV Clinic of Obstetrics and Gynaecology, Silesian Medical Academy, Poland. None of these women had received any hormonal treatment during the three months preceding the laparoscopy. The surgery was performed during an early proliferative phase of the menstrual cycle, directly after menstruation, in order to avoid he possible immunologic effects caused by various hormones on IL-1 production [17]. All the women studied had regular 28–32-day menstrual cycles. Forty-nine women, between 22 and 38 years of age (mean age: 28.2 ± 3.9) had endometriosis, which was histologically confirmed. The endometriosis patients, including early (stage I and II, $n = 38$) and advanced (stage III and IV, $n = 11$) endometriosis, were scored by the revised American Fertility Society (rAFS) classification [18]. The control group consisted of 20 women between 23 and 33 years of age (mean age: 26.8 ± 2.9) affected by non-immunologic infertility. They had no signs of endometriosis or inflammation present in the peritoneal cavity at laparoscopy.

The Ethical Committee of the Silesian Medical Academy approved this study according to the Declaration of Helsinki.

### 2.1. Collection and processing of biologic fluid samples

Peritoneal fluid was aspirated from the posterior cul-de-sac into sterile tubes at the time of laparoscopy. Patients whose PF was contaminated with blood were not included in the study. Fluid samples were processed by centrifuge at 400 × $g$ for 10 min and the clarified PF supernatants were stored at −70 °C until analysis. The peripheral blood sampling was collected by peripheral venipuncture and centrifuged at 1000 × $g$ for 10 min. Serum was aliquoted and stored at −70 °C until the cytokine levels were determined.

### 2.2. Cytokine assay

Peritoneal fluid and serum levels of IL-1α, IL-1 sRII and IL-1 Ra were measured by standard cytokine-specific enzyme-linked immunosorbent assay (ELISA) using commercial kits (R&D Systems, Minneapolis, MN, USA). All measurements were performed in duplicate, according to the manufacturer's instructions, and at the same time for each PF and serum sample to prevent any possible changes due to freezing and thawing. The sensitivity of the kits was approximately 0.5 pg/ml for IL-1α, 10 pg/ml for IL-1 sRII and 22 pg/ml for IL-1 Ra.

### 2.3. Statistical analysis

All results are presented as mean ± S.D. and were examined for normality of distribution by the Shapiro-Wilk test. Parametric data were analysed using an unpaired Student's $t$-test. For nonparametric data differences between groups were analysed using Fisher's exact test (ANOVA), indicative of significance since it analyses the variance relationship both within and among the groups. Correlations were tested by Spearman's rank correlation test and presented as correlation coefficient ($r$). A $P < 0.05$ was considered statistically significant. All analyses were performed with Statistica for Windows 6.0 software.

## 3. Results

Concentrations of the cytokines studied in the PF and serum of women with endometriosis and of those in the control group are shown in Tables 1 and 2.

### 3.1. IL-1α

The results of this study demonstrated the presence of IL-1 in both the PF and the serum of women with endometriosis (mean ± S.D.: 9.8 ± 4.5 pg/ml in PF and 6.7 ± 3.3 pg/ml in serum), and no detectable concentrations of the cytokine in