

**SERUM SOLUBLE UROKINASE-TYPE PLASMINOGEN ACTIVATOR RECEPTOR (SUPAR) AND INTERLEUKIN-6 LEVELS IN ENDOMETRIOSIS****MD Dr. Raziye Desdicioglu\*<sup>1</sup>, MD Melahat Yildirim<sup>1</sup>, MD Gamze Avcioglu<sup>2</sup>, MD Emre Erdem Tas<sup>1</sup> MD Özlem Şengül<sup>1</sup>, MD Ozcan Erel<sup>2</sup> and MD Ayse Filiz Yavuz<sup>1</sup>**<sup>1</sup>Ankara Yildirim Beyazit University, Faculty of Medicine, Obstetrics and Gynecology Department, Ankara, Turkey.<sup>2</sup>Ankara Yildirim Beyazit University, Faculty of Medicine, Biochemistry Department, Ankara, Turkey.**\*Corresponding Author: MD, Dr. Raziye Desdicioglu**

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**ABSTRACT**

**Introduction:** The aim was to compare serum soluble urokinase plasminogen activator receptor (suPAR) and interleukin-6 (IL-6) levels between patients with and without endometriosis. **Materials and Methods:** Twenty two women with endometriosis and 25 healthy women were included in the study. Serum suPAR and IL-6 levels were assessed with the ELISA method. For statistical methods, t test and Receiver Operating Characteristic - ROC analysis were used. **Results:** Serum suPAR level was measured as  $0.38 \pm 0.48$  ng/ml in the endometriosis group and as  $0.16 \pm 0.11$  ng/ml in the control group ( $p < 0.05$ ). The interleukin-6 level was measured as  $11.37 \pm 12.10$  pg/ml in the endometriosis group and as  $4.31 \pm 1.28$  pg/ml in the control group ( $p < 0.05$ ). **Conclusion:** Serum suPAR and IL-6 levels were significantly higher in women with endometriosis. SuPAR may play a role as an inflammatory marker in the pathogenesis of endometriosis.

**KEYWORDS:** Endometriosis, Interleukin-6, soluble urokinase plasminogen activator receptor, Supar.**INTRODUCTION**

Endometriosis is a common gynaecological condition which affects 6-10% of women of reproductive age.<sup>[1]</sup> This disease, which causes chronic pelvic pain and infertility, is characterised by the growth of endometrium-like tissue outside the uterine cavity.<sup>[2]</sup> The annual cost of endometriosis is millions of dollars in the United States alone.<sup>[3]</sup> The cost of the disease is high because its diagnosis is difficult and its treatment is inadequate. Today the disease is diagnosed with the presence of endometrial cells on histopathological examination of tissues obtained by surgical pathology. This invasive diagnostic method delays the diagnosis of the disease.<sup>[4]</sup> Treatment failure is due to recurrent surgical interventions necessary and definitive success cannot be achieved with medical treatment.<sup>[3]</sup> Although studies have been performed with numerous mediators to develop treatments targeting the endometriotic foci directly, specific medical treatment is not yet available.<sup>[3]</sup>

It has been shown that the ectopic endometrial tissue contains different features compared to eutopic endometrium in terms of chemokines, cytokines and other proteins by examination of peritoneal fluid and endometriotic tissue in patients with endometriosis.<sup>[1]</sup> It is believed that these factors contribute to the pathogenesis of the disease. The determination of the relationship of these mediators with definitive diagnosis

and severity of the disease is important for non-invasive diagnosis and definitive treatment of the disease. Various interleukin molecules, tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ) and some adhesion molecules play a role in many events such as proliferation, adhesion and morphogenesis in endometrial cells.<sup>[2]</sup> However, today there is no ideal marker for use in the specific diagnosis of endometriosis.

Interleukin 6 (IL-6) is a multifunctional pro-inflammatory marker which is released from T cells and stimulates B cells. It is a cytokine which plays a role in reproduction, folliculogenesis and the production of steroid hormones.<sup>[2]</sup>

Soluble urokinase plasminogen activator receptor (suPAR) is a soluble form of urokinase-type plasminogen activator receptor (uPAR) which occurs secondary to inflammatory stimuli.<sup>[5]</sup> The plasminogen activation system including suPAR is an important factor in cell adhesion, migration and proliferation in response to inflammation and infection. These molecules are usually released from neutrophils, monocytes, macrophages and activated T cells and serum levels reflect active pathophysiological events on the cell surface.<sup>[6]</sup> The elevated serum suPAR levels are a marker of inflammatory immune activation.<sup>[6]</sup> It was found that an increase in serum suPAR level indicated lipid and carbohydrate metabolism disorders, inflammation and

immune response activation and suPAR is a prognostic factor especially in patients with bacteremia.<sup>[5,7]</sup> Although Supar levels show a positive correlation with other pro-inflammatory markers, it was stated that its diagnostic and prognostic value was higher compared to other pro-inflammatory markers such as interleukin-6 and TNF-alpha and it is not affected by the circadian rhythm and serum freeze/thaw processes<sup>[5-7]</sup>

The purpose of this study is to compare serum soluble urokinase plasminogen activator receptor (suPAR) and interleukin-6 (IL-6) levels among patients with endometriosis and healthy women of the same age and also to evaluate the relationship of these markers to diagnosis and clinical stage of the disease.

## MATERIALS AND METHODS

The present observational prospective study was performed in the Department of Obstetrics and Gynecology at Yildirim Beyazit University, Ataturk Training and Research Hospital, Ankara, Turkey. Necessary approval for our study was received from the Ethics Committee of Ankara Ataturk Training and Research Hospital. Twenty-two eligible women (23–52 years of age) with regular menstrual cycles, who underwent laparoscopy because of primary infertility and chronic pelvic pain, and were subsequently diagnosed with endometriosis after histopathologic confirmation were included in the study. The American Fertility Society revised criteria were used for staging of endometriosis.<sup>[8]</sup> The exclusion criteria were: any gynaecologic pelvic pathology other than endometriosis, such as ovarian tumours or uterine fibroids (diagnosed by clinical examination and pelvic ultrasonography); a history of hormonal therapy or anti-inflammatory drug therapy in the previous month; a postmenopausal status (either physiological or premature); and a previous diagnosis of acute pelvic inflammatory disease. All women with regular menstrual cycles who underwent laparoscopy for tubal ligation during the study period and who showed no evidence of pelvic pathology were enrolled into a control group (n=25). The study protocol was reviewed and approved by the local institutional review board. Written informed consent was obtained from all studied cases and controls. Before laparoscopy, all women provided history and underwent clinical examination in addition to pelvic ultrasonography. On postmenstrual days 8–10, venous blood samples were collected using sterile 10 mL tubes that contained 0.2 mL heparin with a concentration of 1000 IU/mL. All blood samples were centrifuged at 300 rpm for 7 minutes; the clear serum was separated and stored at –80 °C until further processing.

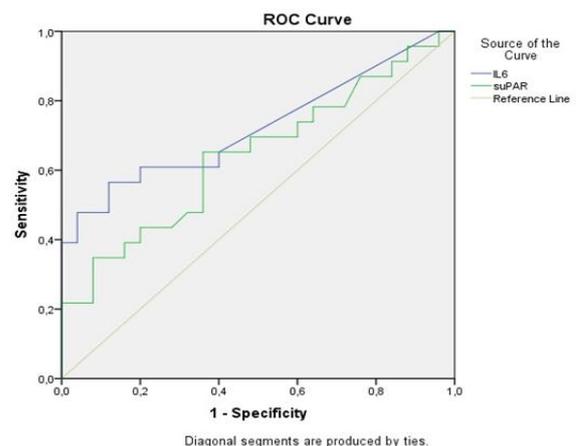
For Supar assays, a micro-ELISA reactive (Receptor (PLAUR/uPAR) ELISA Kit, Hangzhou Eastbiopharm Co. Ltd. Hangzhou, PRC) and a microplate reader (Biotek ELx 800, Bio Tek Instrumentations, Inc, Winooski, VT, USA) were used.

IL-6 levels were measured by using double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) with Human IL-6 Platinum Elisa kit (affymetrix ebioscience).

The t-test was used to statistically evaluate the relationship of the disease with suPAR and IL-6 levels. The relationship between the stage of the disease and suPAR and IL-6 levels in the patient group was investigated with a correlation test. The specificity and sensitivity of suPAR and IL-6 levels in predicting the disease were assessed. The ROC analysis and markers were evaluated to determine if they were sufficient or not in predicting the disease.

## RESULTS

The average age of patients in our study was 36.25±6.38 (23-52) years. There was no difference between the two groups in terms of age, gravidity and BMI. The serum CA 125 level was measured only in the patient group and its average value was found to be 121.25±25.85 IU/ml. Serum suPAR level was measured as 0.16±0.11 ng/ml in the control group and as 0.38±0.48 ng/ml in the patient group (p<0.05). The IL-6 level was significantly higher in the endometriosis group compared to the control group (respectively, 11.37±12.10 pg/ml and 4.31±1.28 pg/ml, p<0.05) (Table. 1). SuPAR and IL-6 levels did not show a correlation between each other and with disease severity. The ROC analysis found suPAR and IL-6 levels are effective in showing the presence of the disease but this effect was found to be more pronounced for IL-6 (Figure 1). According to ROC analysis, the area under the curve (AUC) value was calculated as 0.72 for IL-6 and 0.64 for suPAR.



### Figure legend

Fig. 1: Receiver operating characteristic curve (ROC) for prediction of endometriosis based on the serum levels of interleukin-6 and suPAR measured by enzyme-linked immunosorbent assay. suPAR: Soluble Urokinase – type Plasminogen Activator Receptor, AUC( Area under the curve); suPAR: 0.64.3; IL-6: 0.72.3

**Table 1: Comparison of parameters.**

	Control (25)	Endometriosis (23)
Age (years)	37.15±4.86	35.47±7.47
BMI(kg/m <sup>2</sup> )	26.52±3.93	26.10±3.62
Gravidity(n)	4.21±1.81	1.13±1.14*
IL-6 (pg/ml)	4.31±1.82	11.31±12.10*
suPAR( ng/ml)	0.16±0.11	0.38±0.48*

BMI: Body mass index; IL-6: Interleukin -6; suPAR: Soluble Urokinase – type Plasminogen Activator Receptor \*p<0.05

**Table 2: Diagnostic performance of suPAR and IL-6 for endometriosis.**

Variables	suPAR( ng/ml)	IL-6(pg/ml)
Cut off	0.20	4.28
Sensitivity,%	65	60
Specificity,%	64	80
Area under curve±SE	64.3±0.07	72.3±0.08

**suPAR:** Soluble Urokinase – type Plasminogen Activator Receptor; **IL-6:** Interleukin-6

When 0.20 ng/ml was taken as cut-off, serum suPAR level showed 64% specificity and 65% sensitivity. When 4.28 pg/ml was taken as cut-off, serum IL-6 level showed 80% specificity and 60% sensitivity (Table 2).

## DISCUSSION

In our study, we found that serum IL-6 and suPAR levels were significantly higher in patients with endometriosis compared to the control group. We did not find any other study investigating the relationship between serum suPAR level and endometriosis.

Endometriosis is a condition characterised by persistent lesions and persistent inflammatory reaction.<sup>[1]</sup> It is considered that retrograde menstruation which is the main pathogenetic mechanism of the disease is effective in about 90% of patients. This provides a pro-inflammatory microenvironment for the lesions to become permanent.<sup>[1]</sup> In past studies, the mediators were directly studied in endometriotic foci, peritoneal fluid and serum and the effectiveness of pro-inflammatory mechanisms has been shown in the development of endometriotic foci.<sup>[1-3]</sup>

In previous studies performed on IL-6 levels were higher in the peritoneal fluid of patients with endometriosis compared to the peritoneal fluid of healthy women.<sup>[9]</sup> Moreover, Mosbah, Mihalyi and Kocbek investigated serum IL-6 level in patients with endometriosis and their results are consistent with those in our study.<sup>[2,10,11]</sup> In a study, the impact of CA 125 and IL-6 levels was compared in predicting disease and it was stated that the diagnostic value of IL-6 was low.<sup>[12]</sup> In contrast to our findings, there are also studies showing that IL-6 levels were not associated with endometriosis.<sup>[13,14]</sup> These

results may be caused by the presence of differences in terms of the number of the patients in the study and the disease severity.

SUPAR is a marker which is released by the membrane-dependent plasminogen activator and is associated with immune system activation. It was shown that it was associated with conditions such as many infections, inflammatory diseases and cancer.<sup>[5,7,15,16]</sup> Also it was shown in past studies that suPAR was not affected by the circadian rhythm and serum freeze/thaw processes in contrast to other inflammatory markers.<sup>[5]</sup>

This makes suPAR a highly reliable marker in the diagnosis and the progression of diseases.

There is no other study investigating the relationship between serum suPAR level and endometriosis. In our study, serum suPAR level was found to be significantly higher in the patients with endometriosis compared to the control group but its diagnostic value was lower compared to IL-6 (AUC, respectively, 0.66, 0.71, 0.90). However, in studies of patients with inflammatory disease, suPAR discriminated patients with and without sepsis in an intensive care unit but its diagnostic value was found to be low.<sup>[17]</sup> Moreover, it was found to be higher in patients with bacteremia compared to healthy controls. It was shown that suPAR levels increased within hours in patients with urosepsis.<sup>[5]</sup>

In another study, it was shown that serum suPAR level was higher in male patients with chronic obstructive pulmonary disease compared to a control group.<sup>[15]</sup> Moreover, suPAR was stronger compared to all markers in predicting the risk of mortality in patients in intensive care units.<sup>[5]</sup> In a study investigating the relationship between serum suPAR level and Behcet's disease, serum suPAR levels were found to be higher in the patient group.<sup>[18]</sup> Serum suPAR levels were found to be increased in other inflammatory conditions such as paediatric inflammatory bowel disease and rheumatoid arthritis.<sup>[19,20]</sup> It was found that its serum level was increased in systemic lupus erythematosus and this increase was found to be correlated with organ involvement.<sup>[21]</sup> Serum suPAR levels were examined in ovarian cancer from ovarian pathologies. The increased suPAR level in the premenopausal age group had a high specificity (80%) and sensitivity (95%) to differentiate ovarian cancer and benign ovarian pathologies.<sup>[22]</sup> In a study that examined suPAR levels in the ascitic fluid, there was no significant difference between patients with ovarian cancer and control patients.<sup>[23]</sup>

The limitations of our study are that there was a relatively small number of patients included in our study, there was no comparison between the serum levels before and after surgery, there was no comparison with other ovarian pathologies (such as benign ovarian cysts and ovarian cancer) and peritoneal fluid could not be studied due to technical reasons.

Supar is a relatively new marker and our study is the first study which was performed on endometriosis cases. Consequently, serum suPAR levels were found to be increased in patients with endometriosis and its diagnostic value for endometriosis was found to be lower. A greater number of studies, and if required studies of local peritoneal fluid, investigating the advantageous aspects of suPAR compared to other markers may increase the specificity and sensitivity for non-invasive diagnosis of endometriosis.

The authors declare that there is no conflict of interest regarding the publication of this paper.

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