Efficacy of Angiogenic Factors for Prediction of Endometriosis

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Efficacy of Angiogenic Factors for Prediction of Endometriosis

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ABSTRACT

Efficacy of Angiogenic Factors for Prediction of Endometriosis

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Endometriosis is one of the most common benign gynecologic disorder defined by the proliferation of endometrial tissue outside the uterine cavity. At present, patients with suspected endometriosis must undergo surgery, either exploratory laparotomy or laparoscopy. Direct visualizaion of the lesion and histologic confirmation are essential. The aim of this study is to measure the serum and urinary levels of vascular endothelial growth factors (VEGF), tumor necrosis factor-α (TNF-α), and soluble fms-like tyrosine kinase (sFlt-1) in the patients of endometriosis and to evaluate the efficacy of these angiogenic factors for prediction of endometriosis.

Serum and urine were collected from 30 patients with histology-proven endometriosis (endometriosis group) and from 15 healthy women (control group). Levels of serum and urinary vascular endothelial growth factors (VEGF), tumor necrosis factor-α (TNF-α), and soluble fms-like tyrosine

kinase (sFlt-1) were measured by enzyme-linked immunosorbent assay, and comparison between endometriosis group and control group was made. Urinary angiogenic concentrations were evaluated using both absolute values and those corrected for urinary concentration, by dividing angiogenic factor values by creatinine values. The levels were also evaluated according to the stage of the disease and menstrual cycle. The variables were analyzed using Pearson's correlation coefficient, Student *t*-tests and the Wilcoxon rank-sum test for non-normally distributed data.

The mean age of the patients was 34 years old and the mean parity was 0.65. There was no significant difference in serum levels of angiogenic factors between endometriosis group and control group except TNF-α, which was decreased in endomteriosis group. When urine angiogenic factor levels were corrected for creatinine excretion, urine sFlt-1 levels and TNF-α levels were significantly higher in endometriosis group (median 41.37 pg/mg creatinine for sFlt-1, 0.89 pg/mg creatinine for TNF-α) than control group (median 7.76 pg/mg creatinine for sFlt-1, 0.59 pg/mg creatinine for TNF-α) with $p<0.05$. No significant differences were noted when endometriosis group was analyzed according to the severity of the disease. When analyzed according to the menstrual cycle, the serum VEGF levels and serum TNF-α levels were significantly increased in the secretory phase.

Urinary sFlt-1 and TNF-α are increased in endometriosis patients and may be useful as a new marker for diagnosis of endometriosis.

Key Words: Endometriosis, urine, VEGF, TNF-α, soluble fms-like tyrosine kinase

Efficacy of Angiogenic Factors for Prediction of Endometriosis

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I. INTRODUCTION

Endometriosis is one of the most common benign gynecologic disorder defined by the proliferation of endometrial tissue outside the uterine cavity. The disease is present about 10% of all reproductive-aged women and the prevalence rises to 20%-50% in infertile women.[1] Although endometriosis can be effectively treated, diagnosis can be often confusing. At present, patients with suspected endometriosis must undergo surgery, either exploratory laparotomy or laparoscopy. Direct visualization of the lesion and histologic confirmation are essential. Although laparoscopy is a minimally invasive procedure, it requires general anesthesia and surgical skills. Also, it involves potential complications as well as procedural costs. Thus, a non-invasive diagnostic approach would be beneficial to

both physicians and patients.

The pathogenesis of endometriosis has not been fully understood yet, but the implantation hypothesis is the most widely accepted etiology suggesting that endometrial cells reach peritoneal cavity by retrograde menstruation.[2] These endometrial cells have specific properties that contribute to their implantation and subsequent development into endometriotic lesions. They must escape apoptosis, adhere to mesothelium, proliferate and establish new blood supply. [3] Many studies indicate that endometrial cells in the peritoneal cavity induce an inflammatory response which in turn contributes to lesion maintenance. [4] Angiogenesis appears to be critical and crucial for the peritoneal attachment and development of endometriotic lesions.[5,6] Many studies have focused on the involvement of vascular endothelial growth factor (VEGF) in the serum and peritoneal fluid.[5,7] Also, various angiogenic factors and cytokines including interluekin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), soluble tumor necrosis factor receptor-1 (sTNFR-1), insulin-like growth factor-1 (IGF-1) and angiogenin were found to be increased in the serum and/or peritoneal fluid of endometriosis patients.[8,9] However, no studies have been conducted to evaluate the correlation between serum angiogenic factors and urinary angiogenic factors.

In this study, we measured the serum and the urinary levels of vascular endothelial growth factors (VEGF), tumor necrosis factor-α (TNF-α), and soluble fms-like tyrosine kinase (sFlt-1) in endometriosis patients and healthy women. We evaluated the correlation between the serum and the urinary levels of these angiogenic factors and assessed any of these

markers can discriminate between patients with endometriosis and those without. Such correlation may lead to the discovery of a new non-invasive way to diagnose endometriosis and may allow earlier diagnosis of the disease without surgical confirmation.

II. MATERIALS AND METHODS

1. Patients Selection

Patients were recruited for the study at Department of Obstetrics and Gynecology, YongDong Severance Hospital, Yonsei University College of Medicine, Seoul, Korea. The study was approved by the Institutional Review Board of the YongDong Severance Hospital. A written informed consent was obtained from all subjects enrolled in the study. Patients included in the study all underwent laparoscopy or laparotomy for different indications including pelvic pain, suspicious endometriosis, infertility, and diagnostic evaluation. The serum CA-125 levels were measured for the patients suspicious of endometriosis.. At the time of surgery, possible endometriotic lesions were excised and sent pathology for confirmation. Only after pathologic confirmation, the patient was assigned to the endometriosis group. The extent of endometriosis was determined using the American Society of Reproductive Medicine (ASRM) revised classification.[10] Also, the time of the menstrual cycle was recorded for each patient: proliferative phase, beginning of menses until 14 days before the next menses, and secretory phase, 1-13 days before the next menses.

15 healthy women served as a control group. All women in the control group had regular menstrual cycles (between 21 and 35 days) without menorrhagia nor dysmenorrhea, had not been pregnant for last 3 months,

had not been under hormonal treatment nor using intrauterine device for the last 3 months, and had no abnormal findings by transvaginal ultrasonogram.

2. Preparation of Serum

For control group, blood samples were collected into 10ml sterile tubes containing no additives. For endometeriosis group, blood samples were collected in the same manner before anesthesia. All subjects were abstained from food for at least 8 hours. Samples were immediately centrifuged at 3000rpm for 10 minutes and sediment-free serum samples were obtained. Serum aliquots were frozen at -80℃ until further analysis and thereafter were only thawed once.

3. Preparation of Urine

For control group, urine was collected into sterile plastic tubes. As there is no diurnal variation in the urine VEGF-to-creatinine ratio, no effort was made to obtain samples at a specific time of the day, although most were collected in the morning.[11] For endometriosis patients, urine was collected into sterile plastic tubes when the bladder was catheterized after induction of anesthesia. Samples were immediately centrifuged at 3000 rpm for 10 minutes and sediment-free urine samples were obtained. Just like serum samples, urine aliquots were frozen at -80℃ until further

analysis.

4. Angiogenic Factor Measurements

Serum and urine concentrations of vascular endothelial growth factors (VEGF), tumor necrosis factor-α (TNF-α), and soluble fms-like tyrosine kinase (sFlt-1) were measured using specific commercial sandwich enzyme-linked immunosorbent assays (ELISA) following manufacturer's recommendations (Quantikine, R&D systems Inc, Minneapolis, MN). Urine creatinine was measured with commercial assays at YongDong Severance Hospital. Results were expressed as picogram per milliliter (pg/ml) for serum levels and absolute urine levels, and when the urine levels were corrected for creatinine excretion, they were expressed as picogram per milligram of creatinine (pg/mg Cr).

5. Statistical Analysis

Serum concentrations of VEGF, TNF-α, and sFlt-1 were evaluated with absolute values, and urine VEGF, TNF-α, and sFlt-1 were evaluated using both absolute values and those corrected for urinary concentration by dividing each angiogenic factor by creatinine values. Values in each patient group were analyzed according to the time of menstrual cycle and the severity of the disease.

The SAS system for Windows, version 8.2 (SAS Institute Inc, Cary,

NC) was used for statistical analysis. The relationship between serum and urine values of each angiogenic factors in endometriosis group and in control group were evaluated using Pearson's correlation coefficient. The Wilcoxon rank sum test was used to access differences for non-normally distributed data to compare the distribution of potential confounders within the 2 study groups. Student *t*-tests was also used for comparison of each angiogenic factors and stage of the disease or menstrual cycle phase. Statistical significance was accepted at $P<0.05$.

III. RESULTS

1. Clinical Features

The mean age of the women participated in this study was 33.9 years old for endometriosis group and 30.6 years old for control group. The mean parity was 0.65 for both groups. Of 30 patients with histologic confirmed endometriosis, 6 were classified as stage I, 4 as stage II, 5 as stage III and 15 as stage IV. The mean serum CA-125 level of endometriosis patients was 45.29 ± 37.96 IU/ml. All patients in endometriosis group underwent laparoscopy except one patient who had abdominal wall endometrioma excision.

2. Serum and Urinary VEGF

The median serum VEGF levels of the endometriosis group with range were 172.43 pg/ml and the median urine VEGF levels were 61.09 pg/ml. The median urine VEGF levels corrected for urinary concentration were 103.02 pg/mg Cr. No correlation was noted between serum levels and urine levels or urine-to-creatinine levels. For control group, the median serum levels were slightly higher with 193.68 pg/ml, and the median urine VEGF levels were 82.38 pg/ml. When the urine levels were corrected for urinary concentration, the median urine levels were 45.29 pg/mg Cr. The levels of urine-to-creatinine were much higher in endometriosis group but did not show statistical significance (p=0.15) (Table 1).

Table 1. Serum and Urine VEGF levels

	Endometriosis group	**Control group**	**p value**
Serum VEGF(pg/ml)	172.43(36.44-596.67)	193.68(34.85-396.26)	0.94
Urine VEGF(pg/ml)	61.09(13.54-150.80)	82.38(45.72-188.78)	0.09
Urine-Creatinine VEGF (pg/mg Cr)	103.02(14.56-443.34)	45.29(26.55-115.60)	0.15

Data was expressed as median(range). Wilcoxon rank sum test * P<0.05

3. Serum and Urinary sFlt-1

For sFlt-1, the median serum levels of sFlt-1 in the endometriosis group were slightly lower than the control group (122.79 pg/ml vs. 135.07 pg/ml) without statistical significance. However, the median urinary levels of sFlt-1 in the endometriosis were significantly higher than the control group (33.35 pg/ml vs 12.66 pg/ml, p=0.006) and when corrected for urinary concentration, the median urinary levels were also higher in the endometriosis group with statistical significance (41.37 pg/mg Cr vs 7.76 pg/mg Cr, p=0.001)(Table 2). No correlation was observed between serum levels and urine levels or urine-to-creatinine levels of sFlt-1.

Table 2. Serum and Urine sFlt-1 levels

	Endometriosis group	**Control group**	**p value**
Serum sFlt-1(pg/ml)	122.79(70.58-261.00)	135.07(106.54-163.57)	0.82
Urine sFlt-1(pg/ml)	33.35(4.24-93.44)	12.66(0.03-35.80)	0.006
Urine-Creatinine sFlt-1 (pg/mg Cr)	41.37(4.56-273.66)	7.76(0.02-36.72)	0.001

Data was expressed as median(range). Wilcoxon rank sum test * P<0.05

4. Serum and Urinary TNF-α

The median serum levels of TNF-α were lower in the endometriosis group than the control group as well as the absolute urine levels (0.95 pg/ml vs 1.39 pg/ml, 0.79 pg/ml vs 0.97 pg/ml) with statistical significance. However, when the urine levels were corrected for urinary concentration, the urinary levels of TNF-α were higher in the endometriosis group with statistical significance (0.89 pg/mg Cr vs 0.59 pg/mg Cr, p=0.04) (Table 3).

Table 3. Serum and Urine TNF-α

	Control group	**Endometriosis group**	**p value**
Serum TNF-α(pg/ml)	1.39(0.90-2.50)	0.95(0.79-1.40)	0.001
Urine TNF-α(pg/ml)	0.97(0.90-1.39)	0.79(0.67-0.99)	0.001
Urine-Creatinine TNF-α (pg/mg Cr)	0.59(0.40-1.02)	0.89(0.39-7.87)	0.04

Data was expressed as median(range).Wilcoxon rank sum test * P<0.05

5. Serum and Urine Angiogenic Factors and Severity of the Disease

In the endomteriosis group, each angiogenic factors were evaluated according to the severity of the disease, minimal/mild endometriosis (stage I-II) versus moderate/severe endometriosis (stage III-IV). No significant differences were noted between the severity of the disease and the levels of angiongic factors in serum and urine(Table 5).

Table 4. Serum and Urine Angiogenic Factors and Severity of the Disease

	Mild (I,II)	**Severe (III,IV)**
Serum VEGF pg/ml	223.52 ±167.78	220.82 ± 126.1
Urine VEGF pg/ml	63.35 ± 40.01	73.00 ± 37.39
Serum sFlt-1 pg/ml	141.95±55.07	125.74 ± 30.99
Urine sFlt-1 pg/ml	27.10±11.58	40.30 ± 21.50
Serum TNF-α pg/ml	0.95 ±0.17	1.04 ± 0.12
Urine TNF-α pg/ml	0.80± 0.06	0.81 ± 0.08
VEGF pg/mg Cr	136.61±114.09	112.33 ±108.70
sFlt-1 pg/mg Cr	72.30±81.09	60.14± 61.09
TNF-α pg/mg Cr	1.96±2.17	1.19± 0.94

Data was expressed as mean ± SD. t- test * P<0.05

6. Serum and Urine Angiogenic Factors and Menstrual Cycle in Endometriosis

The levels of the angiogenic factors were also analyzed according to the mesntrual cycle. There were 16 patients in the proliferative phase, and 14 patients in the secretory phase, and the serum VEGF levels and serum TNF-α levels were significantly increased in the secretory phase (p=0.01 and p=0.02 respectively).

Table 5. Serum and Urine Angiogenic Factors According to Menstrual Cycle

	Proliferative	Secretory
Serum VEGF pg/ml	155.02 ± 106.57	283.41 ±133.93 *
Urine VEGF pg/ml	64.14± 35.42	78.55 ± 41.01
Serum sFlt-1 pg/ml	135.44 ± 53.64	129.25±31.77
Urine sFlt-1 pg/ml	30.21 ± 21.94	40.29±17.32
Serum TNF-α pg/ml	0.93 ± 0.010	1.06 ±0.16 *
Urine TNF-α pg/ml	0.80 ± 0.06	0.81± 0.08
VEGF pg/mg Cr	112.06±102.99	137.90±121.64
sFlt pg/mg Cr	66.03± 90.25	65.66±48.39
TNF-α pg/mg Cr	1.55± 1.97	1.44± 1.18

Data was expressed as mean ± SD. t- test * P<0.05

IV. DISCUSSION

Although endometriosis is fairly a common gynecologic disorder, The "gold" standard for diagnosis of endometriosis is still a surgical intervention and therefore, considerable efforts have been made in searching for non-invasive methods, but at present there are no reliable non-invasive techniques nor markers for confirmative diagnosis of this disease, yet.

Many researchers focus on measuring serum concentrations of tumor markers for diagnosis of endometriosis. CA-125 is most extensively studied and most widely used serum marker of endometriosis. [12,13] Some investigators found CA-125 to be elevated, but only in women with more extensive endometriosis. [13,14] Other researchers reported that as a group, women with minimal endometriosis do not have higher circulating levels of CA-125 than women without endometriosis, but by using a cutoff point of 16 U/mL, minimal endometriosis was diagnosed with 71% sensitivity and 71% specificity. [14] However, one potential drawback of the use of CA-125 as a diagnostic test is that it can be elevated in cases of many gynecologic disorders other than endometriosis including ovarian cancer, uterine leiomyomata, pelvic inflammatory disease, and adenomyosis. In addition, the mean CA-125 levels were higher during menses in patients with and without endometriosis. [15]

Recently, the immune system has been shown to play a significant role in the pathogenesis of endometriosis and it is considered by some that

altered immune responsiveness explains why some women develop endometriosis. [16] The observations of immune alterations have led researchers to believe that markers of immune reactivity, particularly cytokines, may be potentially used as a diagnostic marker for endometriosis. The inflammatory response, tissue repair and angiogenesis associated with endometriosis are known to be dependent on the peritoneal macrophage and their secretory products, cytokine. [17] Cytokines are polypeptides or glycoproteins that are secreted into the extracellular compartment mainly by leukocytes. Upon secretion, they exert autocrine, paracrine and sometimes endocrine effects regulating cell proliferation, activation, motility, adhesion, chemotaxis and morphogenesis. Several studies indicate that interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10) and tumor-necrosis factor-α (TNF-α) are associated with pathogenesis of endometriosis.[18-20] Bedaiwy et al. performed a large prospective controlled trial using various cytokines of serum and peritoneal fluid including IL-1β, IL-6, IL-12, IL-13 and tumor necrosis factor- α. They revealed that a threshold of 15 pg/ml for peritoneal TNF-α provide 100% sensitivity and 89% specificity and a threshold of 2 pg/ml for IL-6 provided a sensitivity of 90% and specificity of 67% for diagnosis of endometriosis providing possibility of using immunological markers as a diagnostic marker for endometriosis.[8] However, like CA-125, IL-6 can be elevated in various conditions such as bacterial peritonitis, ovarian cancer and ovarian torsion,

therefore potentially reducing the specificity of the test. [21-23]

Angiogenesis appears to be very critical for the peritoneal attachment and development of endometriosis. During laparoscopy active superficial endometriotic lesions are easily recognized by the abundancy of vasculature. Various cytokines, growth factors and steroid hormones responsible for angiogenesis have been studied by various groups in search for the diagnostic serum markers of endometriosis including IL-1, IL-6, IL-8, epidermal growth factors, fibroblast growth factors, insulin-like growth factors, VEGF and TNF-α.

Vascular endothelial growth factor (VEGF) is one of the most potent and specific angiogenic factors. It is a heparin-binding glycoprotein with dynamic endothelialcells mitogen action and vascular permeability properties. The main biochemical activity of VEGF when it binds to its targeted receptor is that VEGF receptor activation leads to a rapid increase in intracellular Ca2+ and inositol triphosphate concentrations in endothelial cells.[24] The basic physiological function of VEGF is that VEGF-induced angiogenesis allows repair of the endometrium following menstruation. In endometriosis patients, VEGF was localized in the epithelium of endometriotic implants, particularly in hemorrhagic red implants.[25] Moreover, there are increased concentrations of VEGF in PF of endometriosis patients. The exact cellular sources of VEGF in PF have not been precisely defined yet. Although evidence exists to suggest that endometriotic lesions themselves produce this factor, activated peritoneal

macrophages also have the capacity to synthesize and secrete VEGF.[25, 26]

Tumor necrosis factor-α is a pluripotent mediator and angiogenic cytokine that promotes the production of other cytokines in various cells and plays an essential role in the inflammatory process. Because of its importance in other inflammatory processes, it is likely that this cytokine plays a central role in the pathogenesis of endometriosis. Peritoneal fluid TNF-α concentrations are elevated in patients with endometriosis, and some studies show higher concentrations correlate with this stage of the disease. In addition, when mediated by IL-8, it is shown to promote the growth of endometriotic cells.[8,20,27] Activated macrophages play a critical role in the pathogenesis of endometriosis. The secreted TNF-α may play an important role in the local and systemic manifestations of the disease. Because of its importance in other inflammatory processes, it is likely that this cytokine plays a central role in the pathogenesis of endometriosis. [28]

The action of VEGF is mediated by two fms-like tyrosine kinase receptors, Flt-1 (VEGFR-1) and Flk-1 (VEGFR-2), both of which bind the VEGF family with high affinity. Recently, a soluble fms-like tyrosine kinase receptor, sFlt-1, has been discovered. This receptor binds especially VEGF-A with high affinity and has been located in a number of tissues, including human endometrium and placenta.[29,30] This soluble receptor may negatively regulate the action of VEGF on two levels: first, it may bind

and sequester VEGF, thereby reducing its bioavailability; and second, by occupying the VEGF receptors it will prevent VEGF occupancy and the resulting signal transduction.[31]

In this study, we evaluated serum and urine levels of VEGF and TNF-α which are known to be very potent angiogenic factors. We also analyzed soluble fms-like tyrosine kinase 1 (sFlt-1), an antiangiogenic factor, assuming that it would be decreased in endometriosis and loss of balance between VEGF and sFlt-1 might be involved in the pathogenesis of endometriosis.

Although our results showed decreased serum levels of VEGF in the endometriosis group, the urine levels were increased in endometriosis when corrected for urine concentrations. However, the results were not statistically significant, which supports the work done by Potlog-Nahari et al. who reported urine VEGF-A was not increased in endometriosis. [32] However, the fact that urinary VEGF increases in women after hCG stimulation and that it is a marker for certain urinary diseases, including renal cell carcinoma, bladder cancer, minimal change nephrotic syndrome, and chronic renal failure will effect the specificity of the test.[11,33,34] For TNF-α, the serum levels were decreased in the endometriosis group, but the urine levels were increased with statistically significance. One possible explanations for this discripancy is that the serum levels may be increased in a particular phase of the cycle. As shown in our results, TNF-α was increased during secretory phase. There are limited data

regarding variability of serum angiogenic factors during normal menstrual cycle and none regarding the urine factors. In healthy women, endometrial levels of VEGF are greatest in the late luteal phase and during menses, suggesting that serum and urine levels may be greatest at that time.[35] Our data correlate with these findings, showing increased serum VEGF and TNF-α levels in the secretory phase of the menstrual cycle. The serum levels seems to be affected by peritoneal fluid levels which may also vary depending on the menstrual cycle.

One interesting result in our study is concerning the levels of sFlt-1. Resently, serum and urinary sFlt-1 levels were evaluated extensively in preeclapmsia since evidences show that a maladaptive shift in placental production of angiogenic factors such as sFlt-1 may play an important role in the pathogenesis of preeclampsia.[36,67] There is little known about sFlt-1 in endometriosis, and to our knowledge, there is no other studies involving serum and/or urinary sFlt-1 levels in endometriosis. Our results indicated that urinary sFlt-1 levels were increased in endometriosis patients with statistically significances. Although it was not statistically significant, the serum level was slightly decreased. This finding is rather surprising because sFlt-1 is known to be antiangiogenic factor which antagonize VEGF. Hull et al. showed that it could effectively suppressed the growth of ectopic endometrial explants in a nude mouse model of endometriosis.[38] However, Artini et al. also reported high levels of s-Flt1 in the cystic fluid of endometrioma, much higer than the levels of VEGF

suggesting down-regulation of VEGF-mediated angiogenesis in endometriosis.[39] The fact that urinary sFlt-1 was significantly increased in endometriosis group may support the work by Hiratsuka et al. who suggested that Flt-1 has a dual function in angiogenesis, acting in a positive or negative manner in different biological conditions.[40] Further investigation must be done to find out the exact role of sFlt-1 in the pathogenesis of endometriosis.

There is little known about the cyclic variations of urinary angiogenic factors in endometriosis. There is only one study indicating no phase-specific differences in urinary VEGF-A levels in endometriosis patients and none regarding TNF-α and sFlt-1.[32] Since our data indicated increased serum levels of VEGF and TNF-α in the secretory phase, we were expecting similar results in the urine. Although the levels of urinary VEGF were increased in the secretory phase, there was no statistical differences. Our analysis supports the concept that there is no differences in urinary angiogenic factors according to the specific phase of the cycle, although further study with large number of cases are necessary. We also failed to discriminate mild and severe endometriosis using both serum and urinary angiogenic factors. The findings that these measures, especially urinary levels were not significantly affected by the phase of the menstrual cycle makes them more convenient to apply as reliable diagnostic tests in clinical practice. In addition, the levels were not affected by the stage of the disease, hence they should be viewed as

qualitative diagnostic tests, rather than quantitative tests of severity.

The present study showed that the levels of urinary angiogenic factors were increased in the endometriosis and these initial results represent a first step toward the identification of non-invasive potential diagnostic markers for endometriosis. Large clinical trials are needed to confirm these results before clinical applications. Also further investigations should be done on these angiogenic factors, especially sFlt-1, to gain a better understanding of the role and regulation of this cytokine in the pathogenesis of endometriosis.

V. CONCLUSION

We measured the serum and the urinary levels of vascular endothelial growth factors (VEGF), tumor necrosis factor-α (TNF-α), and soluble fms-like tyrosine kinase (sFlt) and evaluated the clinical values of these angiogenic factors for diagnosis of endometriosis.

1. The serum levels of angiogenic factors failed to discriminate endometriosis patients and the control group.
2. The urinary angiogenic factors including sFlt-1 and TNF-α were higher in the endometriosis group with statistically significance. ($p=0.001$, $p=0.04$)
3. No significant differences were noted in the levels of serum and the urinary angiogenic factors when analyzed according to the severity of the disease.
4. Serum VEGF levels and serum TNF-α levels were increased in the secretory phase of the menstrual cycle of endometriosis group. However, no differences was noted among uirnay angiogenic factors.

Our results represent the first evidence to support that angiogenic factors in random urine samples can be used to predict the presence of endometriosis. Further evaluations could lead to development of new non-invasive diagnostic markers for endometriosis.

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ABSTRACT(IN KOREAN)

자궁내막증 진단에 대한 혈관형성인자의 유용성

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조시현

자궁내막증은 부인과에서 흔히 볼 수 있는 질환으로 미국에서 가임 여성의 약 10%에서 이 질병을 접하게 되고 불임 여성의 경우 약 20-50%의 유병율을 나타내고 있다. 그러나 아직까지는 자궁내막증의 원인에 대해서는 확실하게 밝혀진 것은 없다. 최근에는 악성 종양의 전이와 같이 자궁내막세포의 이식세포가 증식하고 신생혈관을 필요로 한다는 가정 하에 자궁내막증에서 혈관형성인자들에 대한 연구들이 다양하게 이루어지고 있으나 아직까지 이런 혈관형성인자들의 혈청, 복강 및 소변내 농도와 질병의 유무나 정도와의 상관관계에 대한 연구는 미비한 상태이며 자궁내막증의 진단에 유용한 표지인자 조차 발견되고 있지 않은 실정이다. 따라서 본 연구에서는 자궁내막증 환자의 혈청 및 소변에서 혈관형성인자들을 측정하고 이를 정상대조군과 비교하여 표지인자로서의 유용성을 평가하고자 하였다.

자궁내막증을 조직학적으로 확진한 환자 30명과 정상군 15명을 대상으로 소변 및 혈청을 채취하여 혈관형성인자인 vascular endothelial growth factors (VEGF), tumor necrosis factor-α (TNF-α), and soluble fms-like tyrosine kinase (sFlt-1)을 ELISA

immunoassay를 사용하여 수치를 측정하여 비교한다. 또한 자궁내막증 환자의 질병의 정도와 생리 주기에 따른 변화를 평가한다.

환자군의 평균 연령은 34세였고 평균 출산력은 0.65였다. 혈청과 소변의 절대 수치는 환자군과 정상군에서 차이가 나타나지 았았으나 소변 수치를 크리아티닌 수치로 보정한 경우, 소변의 혈관형성인자들은 자궁내막증 환자군에서 증가되었으며 특히 TNF-α와 sFlt-1의 경우 정상군에서보다 통계학적으로 의미 있는 상승을 보였다. ($p<0.05$) 자궁내막증 환자의 질병 병기에 따라서 분석한 경우 의미 있는 차이를 보이지 않았고, 환자군의 생리 주기에 따른 변화에서는 분비기에서 혈청 VEGF와 혈청 TNF-α의 의미있는 증가를 관찰할 수 있었다. 자궁내막증 환자에서 소변의 혈관형성인자가 증가되어 있으며 생리주기에 따른 변화가 적어 앞으로 자궁내막증을 진단할 수 있는 비침습적이고 유용한 인자로 사용될 수 있을 것으로 사료된다.

핵심되는 말 : 자궁내막증, 소변, VEGF, TNF-α, soluble fms-like tyrosine kinase