Molecular links between angiogenesis and inflammation in polycystic ovary syndrome

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ABSTRACT

Polycystic ovary syndrome (PCOS) is a cause of infertility in women of reproductive age. Angiogenesis is the physiological process through which new blood vessels form from pre-existing vessels. The aim is to study roles of parameters associated with angiogenesis and inflammation e.g. vascular endothelial growth factor (VEGF), soluble-Fms-like tyrosine kinase (sFlt-1), monocyte chemotactic protein-1 (MCP-1), endostatin and interleukin-18 (IL-18) in PCOS pathogenesis and enlighten possible correlations within PCOS-angiogenesis-inflammation triangle.

A total of 64 women, 31 healthy and 33 with PCOS participated into the study. Their ages, body mass index (BMI) values, routine biochemical parameters were recorded. VEGF, MCP-1, sFlt-1, IL-18 and endostatin levels were determined by ELISA in serum samples. Statistical analyses were performed by SPSS. p<0.05 was accepted as the degree for statistical significance.

Ages, BMI values and BMI distribution profiles of groups did not differ significantly from each other. There were no significant differences between the values detected for MCP-1, IL-18, VEGF and sFlt-1. Upon evaluation of endostatin values, those of patient group were significantly higher than those in control group (p<0.01). Two important correlations were detected (VEGF and MCP-1 as well as VEGF and sFlt-1) in patient group. These were not observed in the control group.

As a result of the literature survey, any study reporting elevated endostatin levels as well as significant correlations between VEGF-MCP-1 and VEGF-sFlt-1 was not found on women with PCOS. In this study, it was concluded that the strikingly elevated values of endostatin in PCOS was a prominent finding.

Keywords: Endostatin, Interleukin-18, Monocyte chemotactic protein-1, Polycystic ovary syndrome, Soluble-Fms-like tyrosine kinase, Vascular endothelial growth factor


INTRODUCTION

Polycystic ovary syndrome (PCOS) is an endocrine disease, which affects the women of reproductive age. Based upon Rotterdam Criteria, this disease is characterized by polycystic ovary morphology, chronic anovulation, oligoamenorrhea, and hyperandrogenism.¹

Angiogenesis is the formation of new blood vessels from pre-existing microvascular channels. Angiogenesis plays some roles in dynamic alterations related to ovaries. Follicle growth and corpus luteum development are due to the multiplication of new capillary vessels. Angiogenesis favors the development of corpus luteum under normal circumstances, then angiogenesis inhibitors cause decreased development of vessels during menstrual cycle. If angiogenesis does not regress but increases, PCOS and infertility may develop.²

Vascular endothelial growth factor (VEGF) is a glycoprotein produced by different cell types as a response to various stimulants. It is one of the mediators responsible for angiogenesis formation. Soluble VEGFR-1/Fms-like Tyrosine kinase (sFlt-1) is the receptor of VEGF. VEGF exerts its effect by way of binding sFlt-1. This binding causes a series of alterations in vascular endothelial cells by activating a certain number of signaling pathways.³,⁴

The most important effect of endostatin is the inhibition of angiogenesis. In vitro studies have shown that it inhibits vessel endothelial cell proliferation as well as migration induced by VEGF. Experimental studies have reported that it reduces tumor formation and development. Endostatin also appears to be a promising biomarker for cardiovascular pathology.⁵,⁶

The levels of interleukin-18 (IL-18), a proinflammatory cytokine, increased in PCOS. It is observed that IL-18 increases cytokine secretion as well as cell interactions by activating effector cells during inflammation and also, ensures activation against pathogens.⁷,⁸

PCOS is one of the most important factors leading infertility. Present reports suggested its relation with angiogenesis. Monocyte chemotactic protein

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Diterima: 2018-06-09
Disetujui: 2019-01-10
publis
MCP-1, VEGF and its receptor sFlt-1 are angiogenesis-related parameters.9,10

The aim of this study is to determine levels of VEGF, sFlt-1, MCP-1, endostatin and IL-18 -thought to contribute to the relations between angiogenesis and inflammation- in patients with PCOS, to compare them with those of healthy controls and to reveal possible associations.

Research and Methods

A total of 64 women -aged between 18-39 years- admitted to Istanbul University, Cerrahpasa Medical Faculty, Department of Obstetrics and Gynecology, In vitro Fertilization Unit were included into the scope of this study. First and second groups comprised 33 women with PCOS and 31 healthy women devoid of chronic diseases. Patients with PCOS were selected based upon Rotterdam Criteria.1 Women with polycystic ovary morphology, chronic anovulation, oligoamenorrhea, and hyperandrogenism were included into the scope of this study.

The study was approved by Istanbul University, Cerrahpasa Medical Faculty, Ethics Committee. Written informed consent forms were obtained from the women participated into the study. Age, height, weight, body mass index (BMI) values were determined. Routine biochemical tests were performed. Information related to the presence of endocrine and chronic diseases such as diabetes mellitus, cardiovascular diseases, hypertension, and also date of admission, treatment protocols confined to patients were recorded. Smokers and alcohol consumers were excluded from the study.

Ten ml. of blood samples were collected from patients and healthy controls within the third day of menstrual cycle. Tests were performed both in Biochemistry Laboratory in Department of Obstetrics and Gynecology and also in Department of Medical Biochemistry. After 30 minutes, blood samples were centrifuged and supernatants were stored at -80°C till the analyses were performed.

VEGF (Human VEGF; e-Bioscience), sFlt-1 (Human sVEGF-R1; e-Bioscience), MCP-1 (Human MCP-1; e-Bioscience), IL-18 (Human IL-18; e-Bioscience) and endostatin (Kit for endostatin. Organism Species: Homo sapiens (Human); Cloud-Clone Corp.) analyses were performed by enzyme-linked immunosorbent assays.

Data obtained were recorded and statistical analyses were performed by SPSSx Statistics Version 20. Data were expressed as mean±SD. Shapiro Wilk test was used to test the normality of the data. The differences between the groups were determined using Mann-Whitney U test. Correlation analyses were performed to determine the associations between the parameters in each group. Statistical degree of significance was determined as p<0.05.

RESULTS

Mean age±SD of control and PCOS groups were 25.0±6.0 years and 26.0±4.5 years, respectively. Corresponding BMI values of the groups were 22.0±3.0 kg/m² and 24.0±4.0 kg/m². Upon evaluation of BMI values, 80% in control group, 70 % in patient group were of 18-25 kg/m² whereas 20% in control group and 30 % in patient group had the values 25.1-35 kg/m². There were no difference between the groups in terms of age and BMI values (p>0.05).

The values and statistical evaluations of the parameters in control and PCOS groups were tabulated in Table 1.

There was no statistically significant difference between the groups in terms of age and BMI values (p>0.05).

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<table>
<thead>
<tr>
<th>Parameter Group</th>
<th>Age (years)</th>
<th>BMI (kg/m²)</th>
<th>MCP-1 (pg/ml)</th>
<th>IL-18 (pg/ml)</th>
<th>Endostatin (pg/ml)</th>
<th>VEGFA (pg/ml)</th>
<th>sFLT (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25.4±6.2</td>
<td>22.4±3.2</td>
<td>86±27</td>
<td>474±232</td>
<td>14939±14556</td>
<td>373±229</td>
<td>0.361±0.112</td>
</tr>
<tr>
<td>median</td>
<td>25.0</td>
<td>21.2</td>
<td>85</td>
<td>515</td>
<td>10142</td>
<td>323</td>
<td>0.344</td>
</tr>
<tr>
<td>min-max</td>
<td>19.0-48.0</td>
<td>18.6-30.1</td>
<td>49-147</td>
<td>139-906</td>
<td>3716-75507</td>
<td>59-1008</td>
<td>0.230-0.840</td>
</tr>
<tr>
<td>PCOS</td>
<td>26.4±4.5</td>
<td>24.1±4.1</td>
<td>91±52</td>
<td>470±238</td>
<td>21166±13285</td>
<td>490±324</td>
<td>0.378±0.099</td>
</tr>
<tr>
<td>median</td>
<td>27.0</td>
<td>23.2</td>
<td>84</td>
<td>510</td>
<td>17673</td>
<td>396</td>
<td>0.352</td>
</tr>
<tr>
<td>min-max</td>
<td>19-35</td>
<td>18.8-32.9</td>
<td>39-235</td>
<td>89-950</td>
<td>6611-68080</td>
<td>88-1248</td>
<td>0.21-0.56</td>
</tr>
<tr>
<td>p</td>
<td>0.124</td>
<td>0.151</td>
<td>0.513</td>
<td>0.814</td>
<td>0.008</td>
<td>0.177</td>
<td>0.317</td>
</tr>
</tbody>
</table>
In PCOS group, two important correlations were calculated between VEGF and MCP-1 ($r=0.411$; $p\leq0.05$) as well as sFlt-1 ($r=0.345$; $p\leq0.05$). None of these correlations were detected in healthy controls (Figure 2).

**DISCUSSION**

Polycystic ovary syndrome is a common and complex endocrine disorder affecting women of reproductive age. From the inflammation point of view, PCOS is reported to be associated with proinflammatory cytokines and chemokines such as tumor necrosis factor-$\alpha$ (TNF-$\alpha$) and IL-6. MCP-1 and IL-18 are also proinflammatory cytokines, however, there are not as many studies on these parameters as the others.\(^7,8,11,12\)

Vascular endothelial growth factor and MCP-1, known as angiogenic factors, play roles in the regulation of endothelial cell functions during angiogenesis, which is a physiological process in which new blood vessels form from pre-existing microvascular channels. Endothelial cells carry VEGF receptor called sFlt-1. There is information about the induction of MCP-1 expression by VEGF.\(^8\) Association of VEGF and MCP-1 as well as elevated VEGF were also reported in patients with lung cancer.\(^9\) This is also confirmed by our findings. In PCOS group, a correlation between VEGF and MCP-1 was obtained. Such a relation was not observed in control group. The association between VEGF and MCP-1 was found as ($r=0.411$ $p\leq0.05$). Our study confirms the positive association of angiogenic factors and the fact that VEGF induces MCP-1.

VEGF shows its proangiogenic effects by binding to some endothelial cell receptors, particularly to its receptor, VEGFR-1, also known as sFlt-1, which is located on vascular system cells. Endostatin directly binds sFlt-1, inhibits angiogenesis by way of blocking VEGF-sFlt-1 interaction to prevent tyrosin phosphorylation of sFlt-1 induced by VEGF (Figure 3).\(^14\)

In a study, no difference was found in sFlt-1 levels between women with PCOS and healthy control group.\(^15\) Our results agree with this finding. There was not any difference between the groups. However, a statistically significant correlation ($r=0.345$ $p\leq0.05$) was found between VEGF and sFlt-1 in the group with PCOS. This positive correlation of VEGF with its receptor sFlt-1 confirms the angiogenesis mechanism in PCOS once more. However, lack of difference between sFlt-1 levels of control and patient groups means that this parameter is not a unique angiogenesis factor.

There are few reports on MCP-1 as well as IL-18, a member of IL-1 superfamily related to this topic. IL-18 is a proinflammatory cytokine and high levels, which are independent of insulin resistance (IR) as well as obesity, were reported in PCOS. Even much more increased levels were observed when both were present in patients.\(^7\) There are other studies, which report positive association between increased IL-18 levels and obesity as well as IR.\(^8,11\)
In our study, groups did not differ from one another from the statistical point of view. This finding may be due to the fact that the difference between BMI values of the groups was statistically insignificant.

In a study, increased MCP-1 levels in women with PCOS were detected compared to women with endometriosis as well as with the infertility of unknown causes. However, a control group was not constituted in this study.

MCP-1 and IL-18 levels exhibited similar trends in our study.

Vascular endothelial growth factor, the member of angiogenic factors family and constitute one of important classes of angiogenic factors, play important roles in the regulation of angiogenesis during follicular growth, ovulation, development of corpus luteum and regression processes. VEGF is the most extensively studied angiogenic factor. It appears to be a marker for progression and remission of neoplastic diseases.

It is secreted by the factors, supporting or inhibiting processes involved in growth of tumor vessels and other complicated mechanisms. VEGF is reported as the most specific and strong supporting factor in this picture.

Many studies reported increased VEGF levels in PCOS however, it is a very recent finding that elevated VEGF levels may be the indicator of early changes arising during the progression of PCOS towards hyperplasia and cancer. In our study, higher values were obtained in patients with PCOS compared to healthy controls however, this alteration was not statistically significant. In our study, VEGF levels were found about 1.3 times higher in PCOS. This finding confirms the PCOS-angiogenesis relationship and the existence of elevated VEGF levels in PCOS.

The only parameter that exhibited significant difference between control and patient groups was endostatin (p≤0.01). This parameter has not been investigated in PCOS before. About 1.4 times higher levels were observed in PCOS compared to controls.

VEGF and endostatin are effective molecules in angiogenesis mechanisms. Therefore, alterations in their levels give important information about angiogenesis. Endostatin is an antiangiogenic factor, however its elevated profile in patients with PCOS refutes the argument advocating that VEGF increase is due to reduction in endostatin.

Statistically insignificant elevation of VEGF in early phases of PCOS may be interpreted as a supporting finding of elevated endostatin levels. Although endostatin level is high, VEGF level did not decrease to normal range. As the disease progresses, VEGF is expected to increase significantly as endostatin decreases. This may be due to the degradation of the balance in angiogenesis mechanisms. The degradation of the balance between VEGF and endostatin may lead to appearance of the problems related to angiogenesis. Angiogenesis mechanisms may return to normal by the help of the antiangiogenic parameter endostatin. The previously unreported findings obtained in our study supports this idea.

The evaluation of these parameters altogether, will be informative for the interpretation of molecular links between inflammation and angiogenesis in PCOS pathogenesis.

Out of endogenous inhibitors of angiogenesis, endostatin takes place at the first rows. Endostatin, a 20kDA C-terminal fragment of Type XVIII collagen, is one of the most powerful inhibitors of angiogenesis. Increased or decreased endostatin levels may be informative for the explanation of pathophysiologic mechanisms of a disease. Recent studies put forward that effects of endostatin are complex and cover more than one mechanism. Endostatin was reported to inhibit endothelial cell proliferation and migration as well as to repress tumor growth.

Endostatin inhibits tumor angiogenesis by preventing the activity of VEGF-related signaling pathways, encourages the efficiency of radiotherapy in oesaphagus cancer, and reverses the immunosuppressive microenvironment associated with the presence of tumors.

Polycystic ovary syndrome incorporates not only abnormalities related to the reproductive system but also a number of cardiometabolic risk factors including endothelial dysfunction and this multifactorial endocrinopathy results in some reproductive and metabolic derangements such as cardiovascular diseases in later years. Endostatin is also evaluated.
as a clinically relevant cardiovascular biomarker. Elevated endostatin levels appear to reflect vascular and myocardial damage, and a worsened prognosis for cardiovascular events or mortality.\(^{6,21-23}\)

To the best of our knowledge, this study was the first to show statistically increased endostatin levels in PCOS in comparison with those in healthy controls. This finding was unique among all the other parameters examined in this study. Also, the significant correlations observed between VEGF and sFlt-1 as well as VEGF and MCP-1 in PCOS group were the first reported findings, which will give light to the matter.

**ACKNOWLEDGMENT**

This study was supported by Istanbul University Rectorate, Scientific Projects Coordination Unit, Project No: 38221. Prof. Dr. Orkide Donma is the executive coordinator of the project.

**REFERENCES**


