Evaluation of Oxidative Stress in Women with Polycystic Ovarian Syndrome as Represented by Serum Ischemia Modified Albumin and Its Correlation with Testosterone and Insulin Resistance

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Abstract

Objective  Ischemia-mediated oxidative stress and inflammation have been reported to be important contributors to the pathogenesis of polycystic ovary syndrome (PCOS). Ischemia-modified albumin (IMA) is a novel marker generated under ischemic and oxidative conditions and may reflect disease activity in distinct disease states. Therefore, we investigated whether the serum IMA levels are affected in infertile PCOS patients.

Methods  Forty-six patients with infertile PCOS, 30 patients with unexplained infertility, and 31 age- and body mass index (BMI)-matched controls were included in this cross-sectional study. Biochemical parameters, serum IMA levels, and their correlations with serum testosterone and insulin resistance were determined for each subject.

Results  In patients with infertile PCOS, the serum IMA levels were significantly elevated (p=0.003) compared with unexplained infertility patients and controls. A correlation analysis suggested that the IMA levels only correlated with the serum free testosterone levels in PCOS patients (r=0.43, p=0.028).

Conclusion  Elevations in the serum IMA levels in infertile PCOS patients may suggest a possible additional role of oxidative stress mechanisms in disease pathophysiology. Moreover, correlation between serum IMA and testosterone levels may influence the quality of oocytes via alterations in the balance of critical follicular fluid factors in the follicular microenvironment.

Key words: polycystic ovary syndrome, unexplained infertility, ischemia-modified albumin, insulin, testosterone

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Introduction

Polycystic ovary syndrome (PCOS) is the most common cause of anovulatory infertility in women and affects an estimated 10% of women of childbearing age (1). It is an important cause of menstrual irregularities ranging from amenorrhea to menorrhagia, as well as androgen excess in women. PCOS is also considered to be an oxidative state, based on decreased antioxidant concentrations, reduced glutathione levels, as well as decreased levels of haptoglobin, a protein with antioxidant properties (2-4). In addition to an association with increased cardiovascular risk due to atherosclerosis, diabetes mellitus, obesity, and metabolic syndrome, oxidative stress is also associated with failed reproductive performance, including infertility, miscarriage, preeclampsia, and diabetes-related congenital malformations (5).

Increasing evidence suggests that reactive oxygen species (ROS) produced during tissue ischemia can generate the
highly reactive hydroxyl radical. The production of this radical can result in N-terminal structural changes in albumin, especially at the N-Asp-Ala-His-Lys sequence, thereby generating ischemia-modified albumin (IMA) (6). Although IMA was originally studied in patients with myocardial ischemia, it has been shown to be useful for evaluating patients with various disease conditions including ischemic events, type 2 diabetes mellitus, pulmonary embolism, liver cirrhosis, coronary bypass surgery, and metabolic syndrome (7-11). Currently, IMA is regarded as a marker of oxidative stress and is also related to ischemia reperfusion in any body organ. In this context, data on IMA in PCOS patients have been obtained (12), but no previous studies specifically address the role of IMA in infertile women with PCOS and unexplained infertility.

This study was performed in order to determine whether serum IMA levels are elevated in women with PCOS and unexplained infertility. Moreover, we also investigated the correlation between testosterone and insulin resistance with serum IMA in both PCOS patients and unexplained infertility patients.

Materials and Methods

Study design

Forty-six PCOS and 30 unexplained infertility patients that received IVF treatment in Dr. Zekai Tahir Burak Women's Health Education and Research Hospital Infertility Center, as well as 31 controls were enrolled in the present study. The study was reviewed and approved by the local ethics committee of the same hospital, and written informed consent was obtained from all patients.

Subjects

PCOS was defined based on clinical, laboratory, and ultrasonography criteria according to the Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop (13). According to this consensus report, a diagnosis of PCOS was considered when at least two of the following criteria were fulfilled:

1. Oligoovulation or anovulation
2. Clinical and/or biochemical signs of hyperandrogenism
3. On ultrasonographic examination, the presence of polycystic ovaries

Unexplained infertility patients recruited for this study were under the age of 40 years with normal ovulatory cycles. Patients also had normal baseline prolactin, follicle-stimulating hormone (FSH), and thyroid-stimulating hormone levels; tubal patency at hysterosalpingography; normal transvaginal ultrasonography; and the presence of both normal ovaries.

Exclusion criteria included patients who had any history of endocrine or systemic disorders. Patients who had used oral contraceptive agents, antihyperlipidemic drugs, hypertensive medications, gonadotropin-releasing hormone agonists and antagonists, glucocorticoids, or antidiabetic drugs within the previous three months were also excluded.

The control group consisted of 31 healthy, age- and body mass index (BMI)-matched, normally ovulating women without any systemic disorders. None of the controls were hirsute, and all had regular menses every 21 to 35 days. All control subjects had normal ovaries in ultrasonographic examination and had normal luteinizing hormone (LH) and FSH levels.

For each patient, the weight and height were measured in light clothing without shoes. BMI was calculated according to the following formula: BMI = weight (kg) ÷ height (m²).

IMA measurement

The cobalt to albumin binding capacity was analyzed using the rapid and colorimetric method defined by Bar-Or et al. (14) Briefly, 200 μL of patient serum were placed into glass tubes, and 50 μL of 0.1% CoCl₂·6H₂O (Sigma-Aldrich, St. Louis, USA) was added to the tubes. This mixture was incubated for 10 minutes to ensure sufficient cobalt-albumin binding. Afterwards, 50 μL of 1.5 mg/mL dithiothreitol (DTT) was added as a coloring agent, and the reaction was quenched 2 minutes later by adding 1.0 mL of 0.9% NaCl. Specimen absorbencies were analyzed at 470 nm by a spectrophotometer. The color of the DTT-containing specimens was compared with that of the colorimetric control tubes. The results are expressed as absorbance units (ABSU). The intra- and interassay coefficients of variation were less than 3.1%.

Biochemical analyses

Blood samples were obtained without using any anticoagulant from a peripheral vein after an overnight fast (10-12 hours) with the participant in a seated position for 30 minutes. The tubes were centrifuged at 3,000 × g for 10 minutes and stored at -80°C until the analysis. As a routine diagnostic work-up, a blood chemistry test including fasting glucose, total cholesterol, triglycerides, FSH, LH, prolactin, insulin, free testosterone and estradiol was conducted on the day of sampling.

HOMA-IR calculation

Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated according to the following formula: fasting insulin (μIU/mL) × fasting glucose (mg/dL)/405.

Statistical analyses

The Statistical Package for Social Sciences (version 18, SPSS Inc., Chicago, USA) for Windows was used to analyze the data. The variables were investigated using visual (histograms, probability plots) and analytical methods to determine whether they were normally distributed. Data are presented as mean ± standard deviation (SD) for normally distributed variables and as median ± (maximum - minimum) for skew-distributed continuous variables. Independent
Table 1. Demographic Characteristics and Biochemical/Hormonal Profiles of the Study Participants.

<table>
<thead>
<tr>
<th></th>
<th>PCOS (n = 46)</th>
<th>Unexplained infertility (n = 30)</th>
<th>Healthy controls (n = 31)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28.1 ± 4.7</td>
<td>29.7 ± 4.8</td>
<td>31.3 ± 6.3</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.5 ± 4.0</td>
<td>26.7 ± 4.8</td>
<td>25.8 ± 4.2</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>88.5 ± 8.4</td>
<td>87.7 ± 9.9</td>
<td>85.9 ± 7.9</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>171.5 ± 36.5</td>
<td>189.0 ± 33.7</td>
<td>182.3 ± 32.1</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>114.3 ± 80.5</td>
<td>137.7 ± 52.4</td>
<td>126.4 ± 64.6</td>
<td>NS</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>2.2 ± 1.8</td>
<td>3.2 ± 2.5</td>
<td>2.9 ± 2.1</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin (µIU/mL)</td>
<td>12.0 ± 4.8</td>
<td>7.2 ± 1.7</td>
<td>6.4 ± 2.3</td>
<td>0.001</td>
</tr>
<tr>
<td>HOMA-IR (%)</td>
<td>3.6 ± 2.2</td>
<td>1.6 ± 0.4</td>
<td>1.8 ± 0.6</td>
<td>0.019</td>
</tr>
<tr>
<td>TSH (µIU/mL)</td>
<td>1.91 ± 1.08</td>
<td>1.90 ± 0.94</td>
<td>1.78 ± 1.2</td>
<td>NS</td>
</tr>
<tr>
<td>FSH (mIU/mL)</td>
<td>6.02 ± 1.23</td>
<td>6.34 ± 1.85</td>
<td>6.22 ± 1.34</td>
<td>NS</td>
</tr>
<tr>
<td>LH (mIU/mL)</td>
<td>7.00 ± 2.82</td>
<td>5.02 ± 2.13</td>
<td>4.98 ± 3.85</td>
<td>0.002</td>
</tr>
<tr>
<td>LH/FSH ratio</td>
<td>1.16 ± 0.47</td>
<td>0.81 ± 0.31</td>
<td>0.84 ± 0.24</td>
<td>0.001</td>
</tr>
<tr>
<td>Free testosterone (pg/mL)</td>
<td>2.48 ± 1.13</td>
<td>1.62 ± 0.46</td>
<td>1.42 ± 0.62</td>
<td>0.009</td>
</tr>
<tr>
<td>Estradiol (pg/mL)</td>
<td>48.86 ± 32.97</td>
<td>44.82 ± 28.90</td>
<td>46.3 ± 31.2</td>
<td>NS</td>
</tr>
<tr>
<td>Prolactin (ng/mL)</td>
<td>16.14 ± 12.51</td>
<td>17.87 ± 13.44</td>
<td>18.9 ± 11.3</td>
<td>NS</td>
</tr>
<tr>
<td>IMA (ABSU)</td>
<td>0.52 (0.21–1.12)</td>
<td>0.33 (0.06–0.99)</td>
<td>0.35 (0.06–0.90)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Values are expressed as either mean ± SD or median (range). ABSU: absorbance units, BMI: body mass index, FSH: follicle stimulating hormone, HDL-C: high-density lipoprotein cholesterol, HOMA-IR: homeostasis model assessment of insulin resistance, hs-CRP: high-sensitivity C-reactive protein, IMA: ischemia-modified albumin, LH: luteinizing hormone, LDL-C: low-density lipoprotein cholesterol, N/A: Not available, NS: not significant, TSH: thyroid stimulating hormone.

Figure 1. Box blot presentation of the serum IMA levels in patients with PCOS and unexplained infertility, and healthy controls.

The mean ages of the women in the PCOS, unexplained infertility, and control groups were 28.1±4.7, 29.7±4.8, and 31.3±6.3 years, respectively. Clinical and laboratory characteristics of patients in all groups are compared in Table 1. Except for serum IMA, insulin, homeostatic model assessment values for insulin resistance (HOMA-IR), free testosterone, LH, and LH/FSH levels, clinical and laboratory parameters were comparable in all three groups. The median serum IMA levels of the PCOS, unexplained infertility, and control groups were 0.52 (0.21–1.12), 0.33 (0.06–0.99), and 0.35 (0.06–0.90) ABSU, respectively. Serum IMA levels of PCOS patients were significantly higher than those of the unexplained infertility and control groups (p=0.003) (Fig. 1).

Although a significant correlation was observed between the serum free testosterone and IMA levels (r=0.43, p=0.028) (Fig. 2), no correlation was found between the serum IMA levels and other clinical and laboratory parameters (Table 2).

Discussion

In this cross-sectional study, we demonstrated that the serum IMA levels are elevated in infertile women with PCOS. The positive correlation between serum IMA and free testosterone levels in PCOS patients added support to the original hypothesis that IMA, as a reflection of oxidative stress in the follicular endocrine microenvironment, may be linked with impaired oocyte developmental competence and embryo quality in association with increased testosterone levels.
The role of IMA in distinct disease states has been a subject of intense research and controversy. Recent studies have demonstrated that significantly higher IMA levels are found in patients with cardiac ischemia, metabolic syndrome, hyperlipidemia, and diabetes mellitus (7-10). Moreover, Ertekin et al. (25) investigated the diagnostic value of IMA levels in patients with acute coronary syndrome and ischemic stroke symptoms and concluded that IMA could be a useful diagnostic marker in acute coronary syndrome and ischemic stroke. In a large series of breast cancer patients, IMA was regarded as a potential novel marker for diagnosing doxorubicin-induced myocardial injury by predicting long-term impairment of cardiac function (26). In contrast, however, Kim et al. (27) reported that IMA levels did not differ between patients with nonischemic chest pain and ischemic chest pain. Those authors therefore proposed that, in clinical practice, assessment of serum IMA levels for triage of patients with acute chest pain does not seem to be useful. Based on this evidence, IMA can be regarded as a
compared with the unexplained infertility group and healthy IMA levels were significantly elevated in PCOS patients. In ST-elevation myocardial infarction patients and no sections of IMA, Dominguez-Rodriguez et al. (31) demonstrated there is little information on diurnal variation in serum levels does not affect serum IMA concentrations. Finally, although run, indicating that skeletal muscle ischemia during exercise did not increase immediately after a marathon patients (29), in a study of marathon runners (30), the serum IMA levels did not increase immediately after a marathon run, indicating that skeletal muscle ischemia during exercise does not affect serum IMA concentrations. Finally, although there is little information on diurnal variation in serum levels of IMA, Dominguez-Rodriguez et al. (31) demonstrated a diurnal fluctuation with significantly higher levels at 02.00 h in ST-elevation myocardial infarction patients and no serum IMA diurnal variations in healthy subjects.

In conclusion, this study has demonstrated that the serum IMA levels were significantly elevated in PCOS patients compared with the unexplained infertility group and healthy controls of similar age and BMI.

The authors state that they have no Conflict of Interest (COI).

References

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