Resistin as a predictor for ovarian response to clomiphene citrate in obese PCOS women

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ABSTRACT

Objective: to evaluate the value of resistin as a predictor of ovulation induction by clomiphene citrate in cases of PCOS.

Design: prospective, controlled clinical study

Setting: kasr Al-Aini Hospital

Materials and Methods: obese subfertile women with PCOS (BMI>30 kg/m2) received clomiphene citrate 50mg / 8hours from the third day of the cycle and for five days. Based on success of ovulation induction, women were divided into two groups: Group I who responded to CC and Group II: those who failed to respond to CC. Blood samples were collected on day 3 of the cycle and resistin, insulin and sugar were assayed.

Results: There was no significant difference between both groups regarding background characteristics, however, there was a significant difference regarding both BMI and resistin between both groups. Multiple linear regression showed a statistically significant value of resistin independent of that of BMI. The ROC curve showed a cut-off value of 4.78 for resistin with area under curve more than 78.9% and sensitivity 66.67% and specificity 82.4% to predict response to clomiphene citrate in obese PCOS women

Conclusion: in obese women, resistin may be of value in prediction of ovarian response to clomiphene citrate. This needs to be confirmed in further trials.

Key words: resistin, PCOS, obesity, clomiphene citrate

Polycystic ovary syndrome (PCO) is a heterogenous syndrome characterized by persistent anovulation, oligomenorrhea or amenorrhea and hyperandrogenism in the absence of thyroid, pituitary, or adrenal disease and is the most common cause of anovulation in adult women.(1). Women with PCOS are frequently insulin resistant and insulin resistance is attributed to have direct effects on ovulation (2). Thus, it seems logical that a marker for insulin resistance could be used to evaluate the response to ovulation induction.

Resistin is a signaling molecule induced during adipogenesis and is secreted by the adipocytes (3). Several reports suggest that resistin plays a causative role in insulin resistance (4). In vitro and in vivo studies have shown that in women with PCOS the sensitivity of insulin to glucose metabolism is subnormal and hyperinsulinemia prevails (5). It now appears that, in most obese patients, obesity is associated with insulin resistance, impaired glucose tolerance and even diabetes (6). Resistin has been implicated in the pathogenesis of obesity-mediated insulin resistance (7). Several studies have investigated the possible cellular mechanism underlying insulin resistance in PCOS employing the major insulin target tissue, the adipocyte (8).
These results showed that insulin-resistance in PCOS represent post-binding defects in signal transduction and may involve defects in more than one step in the signaling pathways (9,10). The aim of the present study was to evaluate resistin as a predictor of ovulation induction by clomiphene citrate in obese women with PCOS.

**MATERIALS AND METHODS**

This is a prospective controlled clinical trial in which obese subfertile women with PCOS (BMI>30 kg/m²) were included. Participants were recruited from the Outpatient Clinic of Gynecology of Kasr El-Aini Cairo University Hospital, among women complaining of infertility and diagnosed as having polycystic ovarian syndrome. The study was done in the period from March 2006 till January 2007 after approval of Local ethical committee.

A full history taking and clinical examination was done. Body Mass Index BMI was calculated as weight (kg)/height(m)². PCOS was defined by clinical, laboratory and ultrasound criteria according to the consensus criteria reported by the Rotterdam group (2004). The clinical criterion was oligomenorrhea (menstrual interval >6 weeks) or amenorrhea (no menstrual loss for 6 months) dating from menarche. The biochemical criteria were increased LH-FSH ratio, normal FSH levels. The ultrasound criteria were enlarged ovaries with an increased stroma and >10 subcapsular follicles of 3–8 mm diameter, arranged peripherally around a dense core of stroma, as described by Adams et al., (1986).

Patients having bilateral tubal block, organic uterine or ovarian pathology, patients with infertile semen analysis of their husbands were excluded. We also excluded women with hypo/hyper-thyroidism, hyperprolactinemia and Cushing syndrome as detected by history, examination and investigations.

All women received clomiphene citrate (Clomid; Global Napi Pharmaceuticals under license of Hoechst Marion Roussel France.) 50mg/8hours from the third day of the cycle and for five days. Transvaginal ultrasonographic serial folliculometry to check ovulation was started from day 8 till ovulation.

Success of ovulation induction was monitored by transvaginal ultrasound done on eighth day of the cycle and every two days. Human chorionic gonadotropin 10,000 IU (HCG; Pregnyl, NV Organon International, The Netherlands.) was administered via intramuscular single injection when the leading follicle reached 20 mm in diameter.

Based on success of ovulation induction, participants were divided into two groups: Group I who responded to clomiphene citrate Group II: those who failed to respond to CC.

**Sample collection**

Fasting blood samples were taken from all women (5 ml) and collected in Vacutainer tubes. All samples were kept at room temperature for at least 30 min to allow the blood to clot and were then centrifuged at 2000 g for 15 min. Serum was collected and stored at -20° C until assayed.

**Hormonal profile**

The concentrations of FSH, LH, estradiol (E2), progesterone (P) and fasting insulin were assayed using reagents supplied by Dpc (Diagnostic products corporation, 5700 west 96th st., Los Angeles) by enzyme chemiluminescence immunoassay.

**Fasting blood glucose**

3 ml of blood on sodium fluoride were collected for estimation of fasting glucose on automated autoanalyzer using reagents supplied by Roche (Roche diagnostics corporation, Indiana Polis, IN, USA.).

**Resistin assay**

Serum resistin was determined by quantitative sandwich enzyme linked immunoassay (ELISA) using kits supplied by Biovendor (Biovendor, LLC, 1459C Sand Hill Road, USA). The serum samples, calibrators and controls were diluted 3 folds with special buffer prior to assay.
Table 1. Comparison between both groups regarding different parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Responsive cases Mean±SD</th>
<th>Non responsive cases Mean±SD</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>28.24±4.81</td>
<td>29.55±4.60</td>
<td>P = .2249</td>
</tr>
<tr>
<td>Duration of infertility(years)</td>
<td>4.53±2.55</td>
<td>5.39±3.12</td>
<td>P = .1974</td>
</tr>
<tr>
<td>BW(Kg)</td>
<td>104.74±12.37</td>
<td>95.93±14.61</td>
<td>P = .0062</td>
</tr>
<tr>
<td>Height(Cm)</td>
<td>163.53±5.65</td>
<td>164.36±4.96</td>
<td>P = .4901</td>
</tr>
<tr>
<td>BMI(Kg/m2)</td>
<td>39.28±5.27</td>
<td>35.58±5.75</td>
<td>P = .0045</td>
</tr>
<tr>
<td>LH(mlu/ml)</td>
<td>10.66±2.08</td>
<td>10.03±2.41</td>
<td>P = .2283</td>
</tr>
<tr>
<td>FSH(mlu/ml)</td>
<td>5.24±3.34</td>
<td>4.53±1.58</td>
<td>P = .4756</td>
</tr>
<tr>
<td>Resistin (ng/ml)</td>
<td>3.99±1.81</td>
<td>9.66±9.75</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>Fasting Glucose(ml/dl)</td>
<td>92.68±21.41</td>
<td>89.24±22.31</td>
<td>P = .4856</td>
</tr>
<tr>
<td>Fasting insulin(µIu/ml)</td>
<td>14.22±13.87</td>
<td>8.11±4.47</td>
<td>P = .1339</td>
</tr>
<tr>
<td>Mean E2 at time of HCG(pg/ml)</td>
<td>358.85±373.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No of follicles &gt;18 mm</td>
<td>1.62±0.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. Progesterone day 22(ng/ml)</td>
<td>12.22±2.45</td>
<td>1.93±1.42</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>4/ 36</td>
<td>0/ 51</td>
<td>P &lt; 0.0001</td>
</tr>
</tbody>
</table>

Statistical evaluation

Data were statistically represented in terms of means and standard deviation (S.D.) where appropriate. For statistical analysis, Comparison between different parameters in the present study was done using Student t test for comparing parametric data. For comparing non parametric data, Chi square ($\chi^2$) test was performed. Yates correction was used instead when the frequency is less than 10. Sensitivity and specificity were generated to estimate the best cut off value using ROC curve (Receiver Operating Characteristic curve). All statistical calculations were done using the computer programs Arcus Quickstat Biomedical version1.0 and Microsoft Excel version 2000.

RESULTS

Eighty seven obese females having PCOS were included in the present study. They were divided into two main groups as evidenced by transvaginal ultrasonography (Group I: 36 women who responded to clomiphene citrate) and Group II (Fifty one women who did not respond to clomiphene citrate). Table 1 shows different characteristics for both groups. There was no significant difference between both groups regarding age, infertility duration, hormonal profile, fasting blood glucose and fasting blood insulin level. However, there was a significant difference regarding both BMI and resistin between both groups. To eliminate the possible confounding effect of BMI, multiple linear regression was done showing a statistically significant value of resistin independent of that of BMI (Table 2).

In the present study, the ROC curve showed a cut-off value of 4.78ng/ml for resistin with area under curve more than 78.9%, the sensitivity was 66.67% and the specificity was 82.4% to predict response to clomiphene citrate in obese PCOS women (Figure 1).

Table 2. Multiple regression analysis of various parameters compared to ovarian response

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistin (ng/ml)</td>
<td>P = 0.0037</td>
</tr>
<tr>
<td>Fasting Glucose(mg/ml)</td>
<td>P = 0.9055</td>
</tr>
<tr>
<td>Fasting insulin(µIu/ml)</td>
<td>P = 0.246</td>
</tr>
<tr>
<td>Age(years)</td>
<td>P = 0.5379</td>
</tr>
<tr>
<td>Duration of infertility</td>
<td>P = 0.7107</td>
</tr>
<tr>
<td>BW(kg)</td>
<td>P = 0.8007</td>
</tr>
<tr>
<td>Height(cm)</td>
<td>P = 0.7266</td>
</tr>
<tr>
<td>BMI(kg/m2)</td>
<td>P = 0.716</td>
</tr>
<tr>
<td>LH(mlu/ml)</td>
<td>P = 0.3772</td>
</tr>
<tr>
<td>FSH(mlu/ml)</td>
<td>P = 0.2979</td>
</tr>
</tbody>
</table>
Polycystic ovary syndrome (PCOS) is associated with an increased incidence of insulin resistance and obesity. Resistin may represent a link between obesity, and these metabolic disorders. Resistin mRNA levels in adipocytes were increased 2-fold in PCOS patients (11).

Also Munir et al. reported that in cultured theca cells resistin enhanced 17 alpha-hydroxylase activity—a marker of ovarian hyperandrogenism in PCOS women—suggesting that the resistin might play local role in the pathogenesis of PCOS. (12) There is also evidence that administration of resistin in mice impaired both glucose tolerance and insulin action, while administration of anti-resistin antibodies improved glucose and insulin action (13). These dates suggest that resistin is a potential mediator of obesity-associated insulin resistance.

In the present study, there was a significant difference in resistin level between women who responded to Clomiphene citrate and those who did not. The role of resistin in PCOS has been investigated in previous studies, which proved that variation in resistin gene promoter was not associated with polycystic ovary syndrome (14). Other investigators reported no difference in serum resistin levels between obese women with PCOS and normal-weight controls (11). However, higher levels of resistin were found significantly in the group of PCOS women with BMI>25 kg/m2, compared with normal weight women with PCOS and BMI<25 kg/m2 and control. A significant correlation between resistin and BMI had also been demonstrated (15).

The previous studies considered normal weight women as the control group whether PCOS or normoovulatory. The worldwide incidence of obesity continues to escalate, despite increased awareness and metabolic disorders such as the development of insulin resistance result from the increasing incidence of obesity (16).

In the present study all cases had PCOS and all of them are obese with BMI >30 but more importantly, is that the comparisons between both groups were based upon their response to ovulation induction, which is a clinically oriented outcome rather than a surrogate biochemical outcome.

We further analyzed the data trying to detect the best cut-off value for resistin by ROC curve. In the era of evidence base medicine, the sensitivity and specificity of a diagnostic tool are of limited use, but should be supplemented with cut-off value. In the present study, it was found that a cut-off of 4.78 ng/ml is of value in prediction of ovarian response to clomiphene citrate.

The present study is an additional step to the available evidence supporting an interrelationship between obesity and PCOS. Resistin can be touted as this link, because it modulates glucose homeostasis, fat homeostasis, influences insulin action, and thus may potentially mechanistically link obesity, insulin resistance and fertility (16).

In conclusion, in obese women, resistin may be of value in prediction of ovarian response to clomiphene citrate. This needs to be confirmed in further trials.

**REFERENCES**


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