N-acetyl cysteine vs. metformin in treatment of clomiphene citrate-resistant polycystic ovary syndrome: a prospective randomized controlled study

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Objective: To compare the effect of N-acetyl cysteine and metformin on hormonal profile (insulin and T) and ovulation rate in women with clomiphene citrate-resistant polycystic ovary syndrome.

Design: Prospective randomized controlled study.

Setting: Department of obstetrics and gynecology in a university hospital in Egypt.

Patient(s): Sixty-one infertile women with clomiphene citrate-resistant polycystic ovary syndrome were assigned randomly to receive either metformin (1,500 mg/d) or N-acetyl cysteine (1.8 g/d) for 6 weeks. Hormonal profile was determined before and after the course of the treatment. Folliculometry was performed to assess ovulation.

Intervention(s): ■ ■ ■ ■

Main Outcome Measure(s): Ovulation rate and insulin and T changes.

Result(s): In the metformin group, there was a significant decrease in the fasting glucose, fasting insulin, and total T. In the N-acetyl cysteine group, there was no significant difference in the fasting glucose or fasting insulin and there was a significant decrease in total T. There was no significant difference in the fasting glucose–fasting insulin ratio in both groups. In the metformin group, the rate of ovulation was 51.6% (16/31), vs. 6.7% (2/30) in the N-acetyl cysteine group, which was statistically significant.

Conclusion(s): Metformin alone is an effective drug in inducing ovulation in clomiphene citrate-resistant polycystic ovary syndrome, whereas N-acetyl cysteine alone is not. Further large studies are required to confirm our results. (Fertil Steril® 2007; ■ ■ ■ ■. ©2007 by American Society for Reproductive Medicine.)

Key Words: Polycystic ovary syndrome, insulin resistance, N-acetyl cysteine, metformin

Clomiphene citrate is the traditional first-line treatment for chronic anovulation that characterizes polycystic ovary syndrome (PCOS) (1). However, 20%–25% of PCOS women fail to ovulate with incremental doses of clomiphene citrate (CC). In addition, clinical data revealed a discrepancy between ovulation rates (75%–80%) and conception rates (30%–40%) during CC treatment (2). For these patients who do not respond to CC, there are a few limited therapies that can be tried before moving on to gonadotropin therapy or laparoscopic ovarian drilling.

Women with PCOS have a greater frequency of insulin resistance and hyperinsulinemia. Recently, a lot of attention has been placed on drugs with insulin-sensitizing action in PCOS patients to induce ovulation in CC-resistant or -nonresistant patients (3, 4). In a systematic review (5), the effectiveness of metformin (MET) in improving clinical and biochemical features of PCOS has been studied, confirming that MET is effective in achieving ovulation in women with PCOS in comparison with placebo.

N-acetyl cysteine (NAC) is the acetylated precursor of both the amino acid l-cysteine and reduced glutathione (6). It has been proven to have activity on insulin secretion in pancreatic cells, as well as on the regulation of the insulin receptor in human erythrocytes (7). In addition, it is a powerful antioxidant and is a potential therapeutic agent in the treatment of cancer and other diseases that are characterized by the generation of free oxygen radicals (8). The peak plasma level of NAC is attained 1 hour after an oral dose, and it disappears from the plasma after 12 hours. The biological activity of NAC is attributed to its sulphydryl group, which enhances glutathione-S-transferase activity, aiding in the protection of all cells and membranes (9). N-Acetyl cysteine commonly is used as a safe mucolytic drug, and at higher doses, it increases the cellular levels of reduced glutathione, an antioxidant, which has been shown to influence insulin receptor activity (10). It has been shown that NAC is able to improve insulin secretion in response to glucose. Moreover, its administration was proposed for the prevention of endothelial damage resulting from oxidant agents in non-insulin-dependent adult diabetic subjects (11).

In 2002, Fulghesu et al. (11) demonstrated that NAC treatment improved insulin sensitivity, T levels, and lipid profile in women with polycystic ovary syndrome. In 2004, Rizk et al. (12) showed that the combination of CC and NAC significantly increased both ovulation and pregnancy rate in women with CC-resistant PCOS. To our knowledge, the role of NAC alone in inducing ovulation in CC-resistant PCOS was never evaluated. Our objective was to compare...
the effect of NAC and MET on hormonal profile (insulin and T) and ovulation rate in women with CC-resistant PCOS.

**MATERIALS AND METHODS**

The present study included 61 infertile patients attending outpatient clinics at Benha University Hospital between December 2004 and December 2005. Institutional review board approval was obtained for this study at Benha University Hospital. Patients were diagnosed as having PCOS according to the Rotterdam criteria for diagnosis of PCOS (14). All patients had previously received CC and were diagnosed as having CC resistance (failure of ovulation after three cycles of CC reaching the dose of 150 mg daily). Our inclusion criteria were as follows: [1] age between 18 and 39 years, [2] period of infertility ≥2 years, and [3] no treatment taken during the last 2 months. Our exclusion criteria were as follows: [1] history of pelvic surgery or infertility factor other than anovulation; [2] endocrinological disorders in the form of hypothyroidism or hyperthyroidism, hyperprolactinemia, and Cushing syndrome, as detected by history, examination, or investigations; [3] patients with organic uterine or ovarian pathology; and [4] patients with hyperglycemia (fasting blood sugar of <100 mg/dL). Hirsutism was diagnosed when the Ferriman-Gallwey score was >8.

Sample size was calculated by using GraphPad Instat software, version 3.01. Twenty-eight patients in each group were required to give the study a power of 80% and α of 0.05. Patients were assigned randomly to receive MET or NAC by using sealed, dark envelopes. A third party (nurse) performed allocation. The patient and the physician monitoring the cycles were blinded to the identity of each medication. Patients underwent physical examination. Weight, height, and waist and hip circumferences were measured. Body mass index was obtained by dividing body weight in kilograms by the square of height in meters. Waist–hip ratio was calculated as the ratio between the smallest circumference of torso (between the 12th rib and the iliac crest) and the circumference of the hip (considered as the maximal extension of the buttocks).

For all patients, the hormonal profile was performed on the 3rd day of the normal cycle or of progestin withdrawal bleeding after fasting for 8 hours. Samples were collected in dry sterile glass tubes, and serum was separated after centrifugation. The serum was divided into two samples; the first sample was analyzed immediately for fasting glucose. The second sample was stored at −20°C until the time of analysis of all samples for hormonal profile (fasting insulin, total T) by using ELISA chemiluminescence. All results were calculated automatically. After 6 weeks, these investigations (fasting glucose, fasting insulin, and total T) were repeated.

A transvaginal ultrasound examination was performed to exclude any pelvic pathology before treatment. Ultrasonography was performed with a transvaginal transducer (6.5 MHz, Siemens). On day 3, each patient underwent a baseline ultrasonographic examination. Metformin (Cidophage tablets, 500 mg per tablet; CID, ARE) was given for 6 weeks from the 1st day of the cycle (in an oral dose of 1,500 mg/d). N-Acetyl cysteine (200 mg per sachet; SEDICO, ARE) also was given for 6 weeks from the 1st day of the cycle (in a dose of 1.8 g by oral administration in three divided doses, 3 sachets per dose). The dose and duration of NAC were chosen based on the study published elsewhere by Fulghesu et al. (11). Transvaginal ultrasound examination was performed every other day from the 10th day of the cycle to diagnose ovulation. Human chorionic gonadotropin (10,000 U, IM; Pregnyl; Organon, Holland) was given when at least one follicle measured 18 mm. Timed intercourse was advised 24–36 hours after hCG injection.

Two days after being given hCG, the patients were assessed for signs of ovulation (disappearance of preovulatory follicle, fluid in the cul-de-sac, and/or corpus luteum formation). Clinical pregnancy was diagnosed when a gestational sac was detected on transvaginal ultrasound examination 1 week after the missed period. Each participant had only one treatment cycle. Sixty-four women were randomized, 32 in each group. Two women discontinued NAC because of nausea and gastrointestinal upset, and one woman in the MET group dropped out.

**Outcome Measures**

The primary outcome was the ovulation rate during the treatment cycle. Secondary outcomes included hormonal profile changes.

**Statistical Analysis**

The proportion of ovulations that occurred in each group was compared by using Fisher's exact test. A P level of <.05 was considered significant.

**RESULTS**

There was no statistically significant difference in age (mean ± SD: 26.73 ± 5.36 y vs. 27.33 ± 3.35 y, P = .74) and duration of infertility (5.07 ± 2.60 y vs. 4.80 y, P = .87) between the groups. Table 1 shows the effect of MET and NAC on clinical characteristics and the hormonal profile.

There were no statistically significant differences in body mass index, waist–hip ratio, menstrual pattern, hirsutism, fasting glucose, fasting insulin, fasting glucose–fasting insulin ratio, and total T between the two groups before treatment. In the MET group, there was a significant decrease in the fasting glucose, fasting insulin, and total T before and after treatment. In the NAC group, there were no significant differences in the fasting glucose or fasting insulin, and there was a significant decrease in total T before and after treatment. There was no significant difference in the fasting glucose–fasting insulin ratio in both groups before and after treatment. In the MET group, the rate of ovulation was 51.6% (16 of 31), vs. 6.7% (2 of 30) in the NAC group, a difference that was statistically significant.
### DISCUSSION

Insulin resistance is a cause of CC failure in patients with PCOS, not only in obese patients but in lean ones as well (15). In addition, hyperinsulinemia may influence ovarian as well as adrenal steroidogenesis. Consequently, insulin-lowering drugs were proven effective in the treatment of patients with PCOS. In the present study, NAC treatment was well tolerated. Two patients reported nausea and gastrointestinal upset. Fulghesu et al. (11) found that NAC administration was well tolerated by all patients and that no adverse effect was observed.

Administration of NAC for 6 weeks in women with CC-resistant PCOS led to no statistically significant changes of fasting glucose or fasting insulin level and to significant lowering of total T level. There was no significant difference in the fasting glucose–fasting insulin ratio before and after treatment. Fulghesu et al. (11) demonstrated that NAC treatment (1.8 g/d for 5–6 wk) was associated with significant increase in insulin sensitivity and reduction in insulin levels, T, and free androgen index in hyperinsulinemic PCOS. In normoinsulinemic and placebo-treated subjects, no significant changes were found. The effect of NAC in hyperinsulinemic PCOS is probably a result of an improvement in insulin receptor activity, leading to a secondary decrease in the \( \beta \)-cell responsiveness to the oral glucose tolerance test (OGTT). The decrease in circulating insulin level was followed by a significant reduction in T levels and free androgen index in patients responding to treatment.

N-Acetyl cysteine has been shown to have diverse biological effects, notably the following: it is antiapoptotic (4), antioxidant (16), protective against focal ischemia (17), and inhibiting of phospholipid metabolism, proinflammatory cytokine release, and protease activity (18). It was suggested that NAC may exert the same effects at the ovarian level and that these activities may be as important as its insulin-enhancing effects in inducing ovulation. However, evidence suggests that in healthy individuals, NAC may act as a pro-oxidant and may lower the GSH and increase the amount of oxidized GSH (19). These results do not support the supposed antioxidant action of NAC.

To our knowledge, the potential reproductive effects of NAC alone never have been evaluated. In the present study, ovulation occurred in 16 patients (51.6%) in the MET group vs. in 2 (6.6%) in the NAC group, indicating the superiority of MET over NAC. N-Acetyl cysteine may be an adjuvant to CC; Rizk et al. (12) showed that the combination of NAC (1.2 g/d) with CC (100 mg/d) for 5 days significantly increased both ovulation and pregnancy rates in obese women.

#### TABLE 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>MET (n = 31)</th>
<th>NAC (n = 30)</th>
<th>P value</th>
<th>MET (n = 31)</th>
<th>NAC (n = 30)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>26.8 ± 1.52</td>
<td>25.8 ± 0.94</td>
<td>.68</td>
<td>25.9 ± 0.97</td>
<td>25.1 ± 1.3</td>
<td>.47</td>
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<tr>
<td>WHR</td>
<td>0.83 ± 0.07</td>
<td>0.82 ± 0.04</td>
<td>.26</td>
<td>0.82 ± 0.08</td>
<td>0.82 ± 0.03</td>
<td>.23</td>
</tr>
<tr>
<td>Menstrual pattern, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oligoamenorrhea</td>
<td>24 (77.4)</td>
<td>23 (76.7)</td>
<td>.37</td>
<td>23 (74.2)</td>
<td>22 (73.3)</td>
<td>.36</td>
</tr>
<tr>
<td>Eumenorrhea</td>
<td>7 (22.6)</td>
<td>7 (23.3)</td>
<td>.19</td>
<td>8 (25.8)</td>
<td>8 (26.7)</td>
<td>.18</td>
</tr>
<tr>
<td>Hirsutism, n (%)</td>
<td>20 (64.5)</td>
<td>18 (60.0)</td>
<td>.19</td>
<td>20 (64.5)</td>
<td>18 (60.0)</td>
<td>.19</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>85.9 ± 9.4</td>
<td>83.3 ± 8.8</td>
<td>.71</td>
<td>81.2 ± 6.2</td>
<td>82.9 ± 9.7</td>
<td>.28</td>
</tr>
<tr>
<td>Fasting insulin (IU/mL)</td>
<td>15.7 ± 3.1</td>
<td>14.5 ± 2.8</td>
<td>.18</td>
<td>12.3 ± 3.4</td>
<td>13.9 ± 4.5</td>
<td>.19</td>
</tr>
<tr>
<td>Fasting glucose-insulin index</td>
<td>5.47 ± 1.80</td>
<td>5.58 ± 2.2</td>
<td>.31</td>
<td>6.1 ± 1.9</td>
<td>5.9 ± 2.7</td>
<td>.208</td>
</tr>
<tr>
<td>Testosterone (pg/mL)</td>
<td>106.47 ± 44.6</td>
<td>98.27 ± 31.5</td>
<td>.08</td>
<td>78.27 ± 30.1</td>
<td>73.87 ± 17.4</td>
<td>.001</td>
</tr>
</tbody>
</table>

Note: Data are mean ± SD unless otherwise indicated. BMI = body mass index; WHR = waist–hip ratio.

- Differences between MET and NAC groups before treatment.
- MET group before and 6 weeks after treatment.
- NAC group before and 6 weeks after treatment.

with CC-resistant PCOS (49.3% vs. 1.3% and 21.3% vs. 0, respectively). These results were not confirmed by other studies. The short duration (5 d only) of NAC administration is not enough to produce the beneficial metabolic and hormonal effects of NAC.

Metformin (a biguanide) has been used to reduce insulin resistance. Although MET appears to influence ovarian steroidogenesis directly, this effect does not appear to be primarily responsible for the attenuation of ovarian androgen production in women with PCOS (20). Rather, MET inhibits the output of hepatic glucose, necessitating a lower insulin concentration and thereby probably reducing the androgen production of theca cells. Metformin also improved fasting insulin levels, blood pressure, and levels of low-density lipoprotein cholesterol. These effects were judged to be independent of any changes in weight that were associated with MET (21).

In the present study, MET treatment was well tolerated; three patients reported minor nausea and vomiting, but intake of MET with meals minimized these side effects; thus, no patients required a discontinuation of the therapy. In the MET group, the rate of ovulation was 53.3%, vs. 6.7% in the N-acetyl cysteine group, which was statistically significant.

Administration of MET for 6 weeks has led to a statistically significant lowering of fasting glucose, fasting insulin levels, and total T. There was no significant difference in the fasting glucose–fasting insulin ratio before and after treatment. Many investigators have demonstrated an improvement in insulin sensitivity and a significant decrease in serum insulin level and free-T level after a short-term treatment with MET for 4–8 weeks (22–24). However, Ehrmann et al. (24) found that hyperinsulinemia and androgen excess in obese nondiabetic women with PCOS are not improved by MET treatment.

These differences may be due to heterogeneity of the populations studied, different methods of assessment of insulin resistance, and variations in duration of MET therapy.

In conclusion, MET alone is an effective drug for inducing ovulation in CC-resistant PCOS, whereas NAC alone is not. N-Acetyl cysteine may be an adjuvant to CC, but not an alternative. Further large studies are required to confirm our results.

REFERENCES

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