Clinical consequences of ovarian stimulation in assisted conception and in PCOS
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N-Acetyl Cysteine is a novel adjuvant to clomiphene citrate in clomiphene resistant polycystic ovary syndrome Patients

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Abstract

Objective: To evaluate the effect of N-acetyl-cysteine (NAC) - a mucolytic drug with insulin sensitizing properties - as an adjuvant therapy in subjects with PCOS resistant to clomiphene citrate (CC).

Design: placebo-controlled, double blind randomized trial.

Setting: University based hospital and private infertility practice.

Patients: One hundred - fifty women diagnosed with CC resistant PCOS, aged 18-39 years undergoing therapy for infertility were included.

Interventions: The patients were assigned randomly to receive either NAC 1.2 gm/day (group I) or placebo (group II) with CC 100 mg/day for 5 days starting at day 3 of the cycle.

Main outcome measures: ovulation rate and pregnancy rate.

Result(s): Combination of CC and NAC significantly increased both ovulation rate and pregnancy rate in women with CC resistant PCOS (49.3% vs 1.3 and 21.3% vs 0% respectively). No cases of ovarian hyperstimulation syndrome were reported in the NAC group. Two cases of miscarriage (12.5%)

Conclusion(s): NAC as an adjuvant to clomiphene citrate was more effective than placebo for CC resistant patients with PCOS. It is safe and well tolerated.
Introduction:

Polycystic ovary syndrome (PCOS) affects up to 10% of women of reproductive age, in which hyperandrogenism, enlarged cystic ovaries, and chronic anovulation often coexist with obesity, hyperinsulinemia, and insulin resistance [1, 2]. Obesity in women with PCOS is rather high, ranging from 30% to 60% [3], whereas hyperinsulinemia is present in more than 50% of patients with PCOS.

Clomiphene citrate therapy has variable success rates in anovulatory women; however it is the lowest in women with PCOS particularly those with insulin resistance. Currently there is increasing evidence that insulin sensitizers are particularly effective in inducing ovulation in PCOS patients [4]. However, not all cases respond to insulin sensitizers [5]. Exploring other mechanisms to induce or augment ovulation in CC resistant patients is a desirable goal in reproductive medicine.

A promising agent is N-acetyl cysteine (NAC). NAC is a safe and well tolerated mucolytic drug that softens tenacious mucous secretions. It is the acetylated precursor of both amino acid L-cysteine and reduced glutathione (GSH)[6]. It has been shown to have proven 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 a potential therapeutic agent in the treatment of cancer, and other diseases characterized by the generation of free oxygen radicals [8]. The peak plasma level of NAC is attained one hour after an oral dose and it disappears from the plasma after twelve 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 & membranes (9).

To our knowledge, the potential reproductive effects of NAC were never evaluated. NAC may be a novel treatment option for augmenting and/or inducing ovulation in patients with chronic anovulation including PCOS. Consequently, the current study was performed to evaluate the effect of NAC administration as an adjuvant to CC on ovulation and pregnancy rates as compared to placebo in patients with CC resistant PCOS.
Materials & Methods
The present study was conducted in a university based hospital and private infertility practice between March 2002 and November 2003. We studied 150 women affected by PCOS, aged 18-39 years. As described elsewhere [10], PCOS was diagnosed by a finding of bilaterally normal or enlarged ovaries (ovarian volume >12 cm3) with the presence of at least 7-10 peripheral cysts per ovary. No patient showed hyperprolactinemia or clinical evidence of hypercorticism or thyroid dysfunction.

All patients had to have at least one patent fallopian tube observed at hysterosalpingography or laparoscopy. The patients' male partners underwent a semen analysis and the results were determined to be adequate according to the latest WHO guidelines.

Eligible patients could not have been receiving any hormonal medications except progesterone for withdrawal bleeding for 2 months before the study. No patient had taken any medication known to affect carbohydrate metabolism for at least 3 months before the study. The body mass index (BMI) was calculated according to the following formula: body weight in kilograms/height in meters squared and obesity was defined as BMI > 30 kg/m2. Informed consent was obtained from each patient before the entry into the study. The study was approved by Benha School of Medicine Institutional Review Board.

Patients who met the inclusion criteria were found to have CC resistance, which was defined as lack of ovulation after treatment with CC, 100 mg, for 5 days in 3 consecutive cycles [11].

Experimental Protocol
Amenorrheic patients began treatment with induction of menses using progesterone in oil, 100 mg. On day 3, each patient underwent a baseline ultrasonographic examination. Clomiphene citrate, 100 mg, was given from day 3 until day 7. In addition to the CC, each patient was selected randomly to receive either NAC (Sedico, Cairo, ARE), in a dose of 1.2 g/day orally, or a placebo (sugar) of the same volume twice daily from day 3 until day 7. Monitoring of the cycle included transvaginal determination of the mean follicular diameter and measurement of serum E2 levels. Monitoring intervals were determined by patient response. Human chorionic gonadotropin was administered when at least one follicle measured 18 mm and the E2 level had raised.
Timed intercourse was advised 24-36 hours after hCG injection. A serum progesterone level was checked on cycle day 21-22. A serum hCG level was determined 14 days after hCG injection if menses had not yet occurred. Pregnancy was defined as a rise in the serum hCG level on serial determinations at least 2 days apart.

Randomization and Blinding
In both groups, patients were randomized to receive CC and either NAC or placebo using sealed envelopes. Each participant had only one treatment cycle. Allocation was done by a third party (nurse). The NAC and placebo were supplied in identical sachets. The patients and the physician monitoring the cycles were blinded to the identity of each medication.

Outcome measures
The primary outcome was the ovulation rate in the treatment cycle. Secondary outcomes included pregnancy rate, number of follicles of ≥ 18 mm, the serum E2 concentration, serum progesterone, endometrial thickness. The major safety end points were the incidence of ovarian hyperstimulation syndrome (OHSS) and multiple gestations. An ongoing pregnancy was defined as a viable pregnancy at least 12 weeks after hCG administration.

Hormonal Assay
Estradiol was measured with a radioimmunoassay (RIA) using direct double-antibody kits (Pantex, Santa Monica, CA). The assay sensitivity was 10 pg/mL. The interassay and intraassay precision of low, middle, and high controls were 14.2% and 16%, 10.6% and 7.9%, and 11.4% and 4.2%, respectively. Follicle-stimulating hormone and Luteinizing hormone were measured with the Flourimetric Enzyme Immunoassay kits (Baxter Diagnostics Inc., Miami, FL). The assay sensitivity of both assays was 0.3 mIU/mL. The interassay and Intraassay precision of low, middle, and high controls were 1.5% and 4.3%, 2.95% and 2.1%, and 3.15% and 3%, respectively, for FSH. For LH, the values were 6.35% and 8.1%, 2.9% and 1.9%, and 2.8% and 2.5%, respectively. Progesterone was measured with an RIA using the antibody coated-tube method (Coat-A-Count; Diagnostic Products Corporation, Los Angeles, CA). The sensitivity of this assay was 0.02 ng/mL.

The interassay precision of low, middle, and high controls for the assay was 8.8%, 3.6%, and 3.9% respectively. Insulin was measured with Axsym insulin diagnostic division...
100 Abott IL MEIA USA ABOTT. The sensitivity of the assay 6-24 mIU/ml. The interassay and intraassay precision of low, middle and high controls were (6-10) (32-48) (96-144) respectively.

**Statistical Analysis**

The proportion of pregnancies that occurred in each group was compared with Fisher's exact test. Comparisons of serum levels between the NAC and placebo groups were analyzed with Student's t-test. A P level of <0.05 was considered significant.

**Results**

A total of 150 patients were randomized to (NAC: n = 75; placebo: n = 75) in a total of 150 CC cycles. As shown in Table 1, there was no difference in age, infertility duration, BMI, W/H, FSH/LH the cycles in which NAC or placebo was given. All participants had BMI more than 25 kg/m2 and the mean BMI in both groups was more than 30 kg/m2 (obese). The mean E2 level and the number of follicles >18 mm at the time of hCG administration in the NAC were significantly higher than the placebo group. Similarly, significantly higher ovulation rates as well as pregnancy rates were noted in the NAC group.

There were five cases of multiple pregnancy in the NAC group. No cases of ovarian hyperstimulation syndrome were reported. There were two cases of miscarriage (12.5%) (one singleton and one multiple pregnancy). On performing subgroup analysis in the NAC group, it was found that at insulin level >20 u/mL; 8 pregnancies (one twin) out of 35 (22.8%) while at insulin < 20 u/mL; 8 pregnancies (three multiple pregnancies) out of 40 (20%) were observed (Odds Ratio = 1.14 95% CI = 0.38 to 3.36).

**Discussion**

Clomiphene citrate failure is a frequent encounter in PCOS patients. Insulin resistance is a cause of CC failure in PCOS patients not only in obese, but lean patients as well [12,13]. In addition, hyperinsulinaemia might influence ovarian as well as adrenal steroidogenesis. Consequently, insulin-lowering drugs were proved effective in the treatment of PCOS patients. The potential insulin-sensitizing properties of NAC in patients with PCOS were recently explored [14,15]. To our knowledge, no previous study focused on the reproductive functions as an end point as a result of NAC treatment in PCOS patients.
Besides its insulin sensitizing effect NAC treatment induced a significant fall in testosterone levels and in free androgen index values [15]. NAC is commonly used as a safe mucolytic drug, and at higher doses it increases the cellular levels of reduced glutathione (GSH), an antioxidant, which has been shown to influence insulin receptor activity [16]. 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 due to oxidant agents in non-insulin-dependent adult diabetic subjects [17].

More recently, it has also been shown to have other diverse biological effects notably: antiapoptotic [18], antioxidant [19], protection against focal ischemia [20], inhibition of phospholipid metabolism, proinflammatory cytokine release, and protease activity [21]. NAC may exert the same effects at the ovarian level and these activities may be as important as its insulin enhancing effects in inducing ovulation.

In the current study, NAC was well tolerated by all the patients and no adverse effects were observed. The results of our study are encouraging; indeed, we obtained a significant increase of both ovulation and pregnancy rates in the NAC group. All participants in our study had only one cycle and this facilitated completion of our study. In addition, the study material was on oral medications of well known tolerability and compliance. The above two factors made this study achieved high level of compliance and completion. Furthermore, no manifestations of OHSS were reported.

Based on the previous hypothesis that NAC treatment is effective only in those patients who were compromised from a metabolic point of view [15], the lack of any positive reproductive outcome in placebo-treated patients further confirmed the effectiveness of NAC administration.

The magnitude of the observed clinical changes is significant from a clinical point of view, especially when compared with previously reported data about the use of metformin or troglitazone [22,23]. In addition, the antiapoptotic effects of NAC [18] may be responsible for the significantly higher number of follicles in the NAC group compared to placebo as it is well known that apoptosis is the main mechanism involved in follicular cohort atresia. Its protective effects against ischemic insults [20] as well as its inflammatory modulating capacity [21] may be additional contributory mechanisms that add to the NAC positive reproductive effects. However, our study was limited to one treatment cycle, whereas the above reported data about other insulin sensitizing agents are the result of 12-24 weeks of
treatment. Moreover, the effects of NAC on the hormonal and metabolic profiles of PCOS patients should be further investigated as other insulin-sensitizing agents do affect both [24].

In conclusion, NAC may be a novel adjuvant treatment for PCOS patients. It is a simple, well tolerated and inexpensive agent. It could be used as an alternative to other insulin sensitizing agents like metformin or troglitazone. The effects of NAC therapy on the hormonal and metabolic profiles, symptoms of hyperandrogenism, and cardiovascular risk factors need further assessment.

Table I: Comparison of the baseline features and clinical outcomes of the 2 treatment groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group I (n=75)</th>
<th>Group II (n=75)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28.9 ±4.7</td>
<td>28.4±5.7</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of infertility(years)</td>
<td>5.0 ±2.9</td>
<td>4.4±2.6</td>
<td>NS</td>
</tr>
<tr>
<td>Wt (Kg)</td>
<td>101.3 ±12.4</td>
<td>99.2±12.3</td>
<td>NS</td>
</tr>
<tr>
<td>Height (m)</td>
<td>164.1 ±5.31</td>
<td>162.5±5.7</td>
<td>NS</td>
</tr>
<tr>
<td>BMI</td>
<td>30.5 ± 2.6</td>
<td>30.1 ± 3.1</td>
<td>NS</td>
</tr>
<tr>
<td>Waist / Hip ratio</td>
<td>0.86 ± 0.05</td>
<td>0.87 ± 0.08</td>
<td>NS</td>
</tr>
<tr>
<td>LH (IU/mL)</td>
<td>10.4±2.2</td>
<td>10.8±2.4</td>
<td>NS</td>
</tr>
<tr>
<td>FSH (IU/mL)</td>
<td>4.7±2.5</td>
<td>5.2±4.8</td>
<td>NS</td>
</tr>
<tr>
<td>LH/FSH ratio</td>
<td>2.2</td>
<td>2.1</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting insulin (U/mL)</td>
<td>18.8 ±4.7</td>
<td>17.2±4.4</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting Glucose (mg/dL)</td>
<td>81.9±12.6</td>
<td>85.9±14.1</td>
<td>NS</td>
</tr>
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<td>E2 at time of HCG(pg/mL)</td>
<td>360.3±367.9</td>
<td>120±10.0</td>
<td>P = .0007</td>
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<td>Ovulation rate</td>
<td>49.3%</td>
<td>1.3%</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>Follicles &gt;18 mm</td>
<td>2.4±0.97</td>
<td>0.01 ± 0.11 *</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>Progesterone</td>
<td>6.87 ± 5.6</td>
<td>1.8 ± 2.2</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>End. Thickness (mm)</td>
<td>5.9±0.7</td>
<td>4.9±1.9</td>
<td>NS</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>16</td>
<td>0</td>
<td>P =0.00006</td>
</tr>
</tbody>
</table>

*only one follicle was shown to be more than 18 mm in one patient
References


13. Moghetti, P., Castello, R., Negri, C., Tosi, F., Perrone, F., Caputo, M., Zanolin, E. and Muggeo, M. Metformin effects on clinical features, endocrine and metabolic
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