N-acetyl-cysteine is a novel adjuvant to clomiphene citrate in clomiphene citrate–resistant patients with polycystic ovary syndrome

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Objective: To evaluate the effect of *N*-acetyl-cysteine (NAC), a mucolytic drug with insulin sensitizing properties, as an adjuvant therapy in subjects with polycystic ovary syndrome (PCOS) resistant to clomiphene citrate (CC).
Design: Placebo-controlled, double-blind randomized trial.
Setting: University-based hospital and private infertility practice.
Patient(s): One hundred fifty women diagnosed with CC-resistant PCOS, aged 18–39 years undergoing therapy for infertility were included.
Intervention(s): The patients were assigned randomly to receive either NAC 1.2 g/d (group I) or placebo (group II) with CC 100 mg/d for 5 days starting at day 3 of the cycle.
Main Outcome Measure(s): Ovulation rate and pregnancy rate (PR).
Result(s): Combination of CC and NAC significantly increased both ovulation rate and PR in women with CC-resistant PCOS (49.3% vs. 1.3% and 21.3% vs. 0%, respectively). No cases of ovarian hyperstimulation syndrome (OHSS) were reported in the NAC group; two cases of miscarriage (12.5%) were reported.
Conclusion(s): The NAC as an adjuvant to CC was more effective than placebo for CC-resistant patients with PCOS. It is safe and well tolerated. (Fertil Steril® 2005;83:367–70. ©2005 by American Society for Reproductive Medicine.)

Key Words: *N*-acetyl-cysteine, polycystic ovary syndrome, clomiphene citrate resistance, pregnancy

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%–60% (3), whereas hyperinsulinemia is present in more than 50% of patients with PCOS.

Clomiphene citrate (CC) 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 patients with PCOS (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.

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A promising agent is *N*-acetyl-cysteine (NAC). It 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 (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 1 hour after an oral dose and it disappears from the plasma after 12 hours. The biological activity of NAC is attributed to its sulfhydryl group, which enhances glutathione-*S*-transferase activity aiding in the protection of all cells and membranes (9).

To our knowledge, the potential reproductive effects of NAC were never evaluated. The NAC may be a novel treatment option for augmenting 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 (PR) as compared to placebo in patients with CC-resistant PCOS.

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MATERIALS AND 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 cm^3) with the presence of at least 7–10 peripheral cysts per ovary. No patient showed hyperprolactinemia, 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 P 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/m^2. 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 three consecutive cycles (11).

Experimental Protocol

Amenorrheic patients began treatment with induction of menses using P-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/d 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 E_2 levels. Monitoring intervals were determined by patient response. Human chorionic gonadotropin was administered when at least one follicle measured 18 mm and the E_2 level had increased.

Timed intercourse was advised 24–36 hours after hCG injection. A serum P level was checked on cycle days 21–22. A serum hCG level was determined 14 days after hCG injection if menses had not yet occurred. Pregnancy was defined as an increase 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 PR, number of follicles of ≥18 mm, the serum E_2 concentration, serum P, and 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 an RIA using direct double-antibody kits (Pantex, Santa Monica, CA). The assay sensitivity was 10 pg/mL. The interassay and intra-assay 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 LH were measured with the fluorimetric enzyme immunoassay kits (Baxter Diagnostics Inc., Miami, FL). The assay sensitivity of both assays was 0.3 mIU/mL. The interassay and intra-assay 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 (Axsym; Abbot, IL). The sensitivity of the assay was 6–24 mIU/mL. The interassay and intra-assay precision of low, middle, and high controls were 6–10 u/mL, 32–48 u/mL, and 96–144 u/mL, 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 <.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, weight/height, and in FSH/LH during the cycles in which NAC or placebo was given. All participants had BMI >25 kg/m^2 and the mean BMI in both groups was >30 kg/m^2 (obese). The mean E_2 level and the number of follicles

TABLE 1

Comparison of the baseline features and clinical outcomes of the two treatment groups.

Variable	Group I (n = 75)	Group II (n = 75)	*P* value
Age (y)	28.9 ± 4.7	28.4 ± 5.7	NS
Duration of infertility (years)	5.0 ± 2.9	4.4 ± 2.6	NS
Wt (kg)	101.3 ± 12.4	99.2 ± 12.3	NS
Height (m)	164.1 ± 5.31	162.5 ± 5.7	NS
BMI	30.5 ± 2.6	30.1 ± 3.1	NS
Waist/hip ratio	0.86 ± 0.05	0.87 ± 0.08	NS
LH (IU/mL)	10.4 ± 2.2	10.8 ± 2.4	NS
FSH (IU/mL)	4.7 ± 2.5	5.2 ± 4.8	NS
LH/FSH ratio	2.2	2.1	NS
Fasting insulin (U/mL)	18.8 ± 4.7	17.2 ± 4.4	NS
Fasting glucose (mg/dL)	81.9 ± 12.6	85.9 ± 14.1	NS
E_2 at time of hCG (pg/mL)	360.3 ± 367.9	120 ± 10.0	.0007
Ovulation rate	49.3%	1.3%	<.0001
Follicles >18 mm	2.4 ± 0.97	0.01 ± 0.11[a]	<.0001
Progesterone	6.87 ± 5.6	1.8 ± 2.2	<.0001
Endometrial thickness (mm)	5.9 ± 0.7	4.9 ± 1.9	NS
Pregnancy	16	0	.00006

[a]Only one follicle was shown to be more than 18 mm in one patient.

Rizk. Use of N-acetyl cysteine in patients with PCOS. Fertil Steril 2005.

>18 mm at the time of hCG administration in the NAC group were significantly higher than the placebo group. Similarly, significantly higher ovulation rates as well as PRs were noted in the NAC group.

There were five cases of multiple pregnancies in the NAC group. No cases of OHSS 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 μ/mL there were 8 pregnancies (one twin) of 35 (22.8%), whereas at insulin <20 μ/mL, 8 pregnancies (three multiple pregnancies) of 40 (20%) were observed (odds ratio = 1.14, 95% confidence interval [CI] = 0.38–3.36).

DISCUSSION

Clomiphene citrate failure is a frequent encounter in patients with PCOS. Insulin resistance is a cause of CC failure in patients with PCOS, not only in obese, but in lean patients as well (12, 13). In addition, hyperinsulinemia might influence ovarian as well as adrenal steroidogenesis. Consequently, insulin-lowering drugs were proved effective in the treatment of patients with PCOS. 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 patients with PCOS.

Besides its insulin-sensitizing effect NAC treatment induced a significant decrease in T levels and in free androgen index values (15). The NAC is commonly 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 (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). The 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. We obtained a significant increase of both ovulation and PRs in the NAC group. All participants in our study had only one cycle and this facilitated completion of our study. In addition, the study participants were on oral medications of well-known tolerability and compliance. These two factors made this study achieve a 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 the contributory mechanisms that added to the NAC positive reproductive effects. However, our study was limited to one treatment cycle, whereas the 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 patients with PCOS should be further investigated as other insulin-sensitizing agents do affect both hormonal and metabolic features (24).

In conclusion, NAC may be a novel adjuvant treatment for patients with PCOS. 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.

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