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N-acetylcysteine as an adjuvant to clomiphene citrate for successful induction of ovulation in infertile patients with polycystic ovary syndrome

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Abstract

Aim: The aim of this study was to evaluate the effect of oral N-acetylcysteine (NAC) administration as an adjuvant to clomiphene citrate (CC) on induction of ovulation outcomes in patients with polycystic ovary syndrome (PCOS).

Material and Methods: In this placebo-controlled double-blind randomized clinical trial, 180 PCOS infertile patients were randomly divided into two groups for induction of ovulation. Patients in group 1 received CC 100 mg/d plus NAC 1.2 g/d and patients in group 2 received CC plus placebo for 5 days starting at day 3 of the cycle. On the 12th day of the menstrual cycle in the presence of at least one follicle with an 18–20-mm diameter in ultrasound evaluation, 10 000 U hCG was injected intramuscularly and timed intercourse was advised 36 h after hCG injection. Serum β-hCG level was measured on the 16th day after hCG injection.

Results: The number of follicles >18 mm and the mean endometrial thickness on the day of hCG administration were significantly higher among the CC+NAC group (*P*-value = 0.001). The ovulation and pregnancy rates were also significantly higher in the CC+NAC group (*P*-value = 0.02 and 0.04, respectively). No adverse side-effects and no cases of ovarian hyperstimulation syndrome were observed in the group receiving NAC.

Conclusion: NAC as a safe and well-tolerated adjuvant to CC for induction of ovulation can improve the ovulation and pregnancy rates in PCOS patients. It may also have some beneficial impacts on endometrial thickness.

Key words: clomiphene citrate, N-acetylcysteine, ovulation, polycystic ovary syndrome, pregnancy rate.

Introduction

Polycystic ovarian syndrome (PCOS) is the most common endocrinopathy in women of reproductive age, with a prevalence of up to 10%.[1] The diagnosis of PCOS is determined by the presence of two of the following conditions: oligo-ovulation or anovulation, hyperandrogenemia, hyperandrogenism and polycystic ovaries detected by ultrasonography.[2] The three most bothersome symptoms commonly reported by affected women are hirsutism, irregular periods, and infertility.[3] A significant proportion of patients with PCOS have been found to suffer from defective insulin secretion and insulin resistance[4] and hyperinsulinemia is present in more than 50% of patients with PCOS.[5]

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Clomiphene citrate (CC), the traditional first-line medication for induction of ovulation in anovulatory women, has variable success rates; however, its success rate is the lowest in women with PCOS and insulin resistance. There is evidence indicating that insulin sensitizers decrease hyperandrogenism, and hyperinsulinemia in patients with PCOS, and are particularly effective for induction of ovulation among these patients.[6–8]

N-acetylcysteine (NAC), which is commonly used as a safe mucolytic drug, has been shown to influence both the insulin secretion in pancreatic β-cells, as well as the regulation of insulin receptors in human erythrocytes.[9] NAC has antioxidant effects via increasing the cellular levels of reduced glutathione,[10,11] and its preventive effect on the endothelial damage in non-insulin-dependent diabetic subjects has also been proved.[12] In recent years a limited number of studies has shown the possible benefits of NAC administration in improving insulin sensitivity and better induction of ovulation outcomes in patients with PCOS.[13,14]

This study was conducted to evaluate the effect of oral NAC administration as an adjuvant to CC on induction of ovulation outcomes in PCOS patients.

Methods

This placebo-controlled double-blind randomized clinical trial was conducted in Shahid Beheshti University of Medical Sciences IVF center, Taleghani Hospital, between January 2008 and December 2009. All infertile women referred to our center with PCO (based on Rotterdam criteria, ESHRE/ASRM 2004),[2] aged 20–35 years, infertility duration less than 10 years, body mass index (BMI) < 35 kg/m^2, both patient tubes confirmed by hysterosalpingography or laparoscopy and with partner's normal semen analysis results (total volume > 2cc, concentration > 20 million/ml, total motility > 50%, normal morphology > 14%) were included in the study. Patients with thyroid dysfunction, hyperprolactinemia, hypercorticism, history of large ovarian cyst formation (>6 cm), history of visual disturbance caused by CC and finally history of asthma and or allergy to medications were excluded from the study. Patients who had received any hormonal medications (except progesterone for withdrawal bleeding) or medications affecting glucose metabolism for at least 3 months before the study were also excluded. Also, none of the patients or their male partners had any sexual dysfunction interfering with successful intercourse. The sample size was calculated assuming the true ovulation rate for (CC+NAC) and (CC+Placebo) to be 52.1% and 17.9%, respectively.[15] Based on these assumptions, the total sample size of 160 patients was estimated to provide 80% power to detect the difference of 20% at the 0.05 level of significance. Taking into consideration a 10% dropout rate, the required minimum number of enrolled patients was determined to be 180 (90 group 1, 90 group 2). All participants gave written informed consent before entering the study. The study protocol was approved by the ethics committee of Shahid Beheshti University of Medical Sciences.

On the 3rd day of the menstrual cycle (induced by 200–300 mg progesterone-in-oil injection in amenorrheic patients) a baseline vaginal ultrasound examination and serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid-stimulating hormone and thyrotropin (TSH) and prolactin levels assessment (all measured by immunoreactive multianalysis) were performed for all patients who were candidates for induction of ovulation. Then patients were randomly divided into two groups. In the first group, patients received 100 mg CC plus 1200 mg NAC from day 3 until day 7 of the menstrual cycle. NAC was given to the subjects in the form of powder inserted in small pockets to be diluted into one standard glass of water and taken orally in two daily divided doses. In the second group in addition to 100 mg daily CC, patients received a placebo (oral rehydration solution [ORS] powder)) from day 3 until day 7. ORS powder was given to the subjects in the same pockets as NAC for two daily divided doses.

On the 12th day of the menstrual cycle, patients were monitored by transvaginal ultrasound examination to evaluate the mean follicular diameter and the endometrial thickness. In the presence of at least one follicle with 18–20 mm in size, 10 000U hCG was injected intramuscularly and timed intercourse was advised 36 h after hCG injection. Serum β-hCG level was measured on the 16th day after hCG injection. With two serial positive β-hCG levels (at least 2 days apart) another transvaginal ultrasound examination was performed on the 6th week of gestation to determine the clinical pregnancy.

Data were analyzed using SPSS. The significance of the difference between experimental and control groups for continuous data was assessed using the Student's *t*-test. Fisher's exact test was used to compare the categorical data. A *P*-value < 0.05 was considered to be statistically significant.

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Table 1 Baseline variables and induction of ovulation outcomes in the case and control groups

Variable	CC+NAC ($n = 82$)	CC+Placebo ($n = 85$)	*P*-value
Age (years)	27.22 ± 3.32	27.41 ± 3.41	NS
Duration of infertility (years)	4.39 ± 1.96	4.45 ± 1.94	NS
BMI	26.78 ± 2.24	26.67 ± 2.01	NS
LH/FSH ratio	1.45 ± 0.63	1.40 ± 0.61	NS
Follicles (>18 mm)	1.5 ± 0.89	1 ± 0.77	0.001
Endometrial thickness (mm)	6.6 ± 1.69	5.4 ± 1.61	0.001
Ovulation rate	45.12%	28%	0.02
Pregnancy rate	20.73%	9.4%	0.04

BMI, body mass index; CC, clomiphene citrate; FSH, follicle-stimulating hormone; LH, luteinizing hormone; NAC, N-acetylcysteine; NS, not significant.

Results

A total of 180 patients were randomly divided into two groups (group one CC+NAC [$n = 90$], group two CC+Placebo [$n = 90$]). Eight patients in the first group and four patients in the second group exited the study due to inappropriate drug intake or discontinued cycle. There were no statistically significant baseline differences between the two groups in age, duration of infertility, BMI and LH/FSH ratio of patients (Table 1). As shown in Table 1, the number of follicles more than 18 mm in size and the mean endometrial thickness on the day of hCG administration were significantly higher among patients who received CC+NAC compared to those patients who received CC+Placebo (*P*-value = 0.001). Also, the rates of ovulation and pregnancy were significantly higher in the CC+NAC group (*P*-value = 0.02 and 0.04, respectively).

There was one case of twin pregnancy in the CC+NAC group. Whereas, one case of twin pregnancy and one case of triple pregnancy were observed in the CC+Placebo group. One cycle was canceled in the later group because of formation of a 5 × 5.6-cm ovarian cyst, but no case of ovarian hyperstimulation syndrome (OHSS) was observed in either group. None of the patients represented any other possible adverse effects of CC or NAC.

Discussion

Based on our data we have found a significantly better ovulation rate in PCOS patients who received NAC as an adjuvant to CC for induction of ovulation. Since the insulin resistance has been shown to be a cause of CC failure in both obese and non-obese PCOS patients,[16,17] the potential insulin-sensitizing effects of NAC may lead to better induction of ovulation in these patients.[13,18] Through acceleration of glutathione synthetase hormone synthesis, increased levels of glutathione (an important antioxidant), inhibition of oxidative stress and consequently preservation of insulin receptors against oxidant agents, NAC probably influences insulin receptor activity and results in an increase of cellular glucose consumption which is an indicator of the insulin sensitivity state.[13,18–20]

In a study by Fulghesu *et al*., NAC administration significantly reduced the insulin area under the curve after OGTT and increased the peripheral insulin sensitivity.[13] A significant fall in testosterone level and free androgen index was also demonstrated with NAC treatment in PCOS patients in their study.[13] Kilic-Okman *et al*. have described NAC as an effective medication for reducing serum insulin and testosterone levels and improving the homocysteine status as well as lipid profiles among PCOS patients.[21] In our previous study, we also observed a significant decrease in weight, BMI, waist/hip ratio, fasting blood sugar, serum insulin, total cholesterol, low-density lipoprotein (LDL) levels, and homeostasis model assessment insulin resistance (HOMA-IR) index after a 6-week treatment with NAC in PCOS patients.[18] High-density lipoprotein (HDL) levels were also elevated significantly and NAC improved the lipid profile, hormonal levels, and ovulation status in women with PCOS in that study.[18] It has also been shown that prolonged treatment with NAC plus L-arginine might restore gonadal function in PCOS in association with an improvement in insulin sensitivity.[22] Further studies are required to evaluate the beneficial effects of NAC on hormonal and metabolic profiles of PCOS patients in comparison with other insulin-sensitizing agents, such as metformin.

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In addition to its insulin-sensitizing and androgen-reducing effects, some other biological effects of NAC, such as anti-apoptotic and antioxidant effects,[23,24] inhibition of phospholipid metabolism, proinflammatory cytokine release, and protease activity,[25] may lead to better folliculogenesis and ovulation rate in PCOS patients.

To our knowledge, only a limited number of studies have evaluated the induction of ovulation outcomes in PCOS patients treated with NAC. Elnashar *et al*. showed that NAC is not an effective remedy to induce ovulation in CC-resistant PCOS patients,[26] but another study by Badawy *et al*. noted that compared to placebo, the addition of NAC to a CC regimen in patients with PCOS would increase ovulation rates significantly.[15] A recent study has found significant increase in ovulation, pregnancy rates and better reproductive outcome in PCOS patients who received NAC after unilateral laparoscopic ovarian drilling.[27] Rizk *et al*. also found that a combination of CC and 1.2 g/d NAC for induction of ovulation significantly increases the E2 level at the time of HCG administration, ovulation and pregnancy rate in women with CC-resistant PCOS compared to the CC plus placebo group.[14] Our data support the results of their study, but based on our findings, NAC may be beneficial as an adjuvant to CC for induction of ovulation in a more expanded range of PCOS patients and not just limited to CC-resistant PCOS women. No adverse effects of NAC were observed among PCOS patients in both studies, and the drug seemed to be safe and well-tolerated by all patients.

It has been demonstrated that CC may have a negative impact on the quality and quantity of cervical mucus and endometrial development that may cause implantation failure, luteal phase defects and significant thinning of the endometrium, in a dose-dependent manner.[28–30] These adverse effects of CC on the endometrium may explain in part the relatively poor pregnancy rates associated with CC despite the high rate of ovulation.[31] In contrast to the study by Rizk *et al*., which did not reveal any significant change in endometrial thickness,[14] in our study a significant improvement of endometrial thickness in PCOS patients who received NAC as an adjuvant to CC was observed. In this case, NAC may also improve the implantation rate by increasing endometrial thickness in PCOS patients receiving CC. The antioxidant effects of NAC and its protective characteristics against focal ischemia have been demonstrated in previous studies[23,32] which might be a possible mechanism for NAC's positive impact on endometrial thickness. Further studies using Doppler ultrasound are required to show the possible benefits of NAC on endometrial growth and the implantation rate.

In conclusion, based on our data, NAC as an adjuvant to CC for induction of ovulation can improve the ovulation and pregnancy rates in PCOS patients and may also have some beneficial impacts on endometrial thickness. NAC is well-tolerated, safe, and inexpensive and may be a novel adjuvant treatment to improve the induction of ovulation outcomes in PCOS patients.

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