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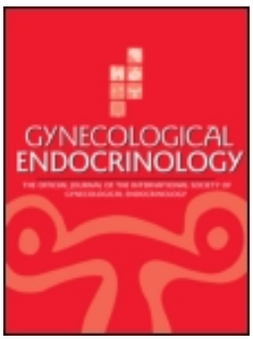

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PCO

The adjuvant effect of metformin and *N*-acetylcysteine to clomiphene citrate in induction of ovulation in patients with *Polycystic Ovary Syndrome*

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Abstract

Objectives: To assess the adjuvant effect of metformin and *N*-acetylcysteine (NAC) to clomiphene citrate (CC) in induction of ovulation in Polycystic Ovary Syndrome (PCOS) patients.
Study design: 120 women with PCOS were randomly divided into three equal groups: group I received CC only, group II received CC plus NAC and group III received CC plus metformin.
Results: There was a significant difference between group II and other two groups regarding average number of ovulatory follicles >18 mm (2.25 versus 1.75 and 1.89, respectively), but no significant difference between the three study groups regarding number of intermediate follicles 14–18 mm (4, 10 and 4, respectively). There was no significant difference between the three study groups regarding occurrence and laterality of ovulation, pregnancy rate per cycle but a significant difference between group II and other two groups regarding pregnancy rate per patient (20% versus 10% and 10%, respectively, *p* value 0.05). There was a highly statistically significant difference between group II and other two groups regarding peak endometrial thickness (7.3 ± 1.1 versus 5.4 ± 0.6 and 5.3 ± 0.6, respectively).
Conclusions: NAC as an adjuvant to CC for induction of ovulation improves ovulation and pregnancy rates in PCOS patients with beneficial impacts on endometrial thickness.

Keywords

Clomiphene citrate, induction of ovulation, metformin, *N*-acetylcysteine, Polycystic Ovary Syndrome

History

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Introduction

The Polycystic Ovary Syndrome (PCOS) is a heterogeneous disorder, whose principal features include androgen excess, ovulatory dysfunction, and/or polycystic ovaries, and is recognized as one of the most common endocrine/metabolic disorders of women [1].

It is believed to affect about 5–10% of all women. PCOS is a collection of signs, symptoms and endocrine disturbances [2].

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 and are particularly effective for induction of ovulation among patients with PCOS [3].

Combining metformin and CC give higher rates of regular cycles, ovulation success and conception when compared with treatment with CC alone [4].

The antioxidant effects of *N*-acetylcysteine (NAC) and its protective characteristics against focal ischemia have been demonstrated in previous studies which might be a possible mechanism for NAC's positive impact on endometrial thickness [5].

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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, inhibition of phospholipid metabolism, proinflammatory cytokine release and protease activity, may lead to better folliculogenesis and ovulation rate in PCOS patients [6].

There are insufficient data about combining therapy of NAC and CC in PCOS women.

This study was conducted to assess the adjuvant effect of metformin and NAC to CC in induction of ovulation in patients with PCOS.

Material and methods

This prospective randomized study was conducted at Kasr El Aini Hospital, Cairo University, Egypt, in the period from September 2012 to March 2014. After approval of local ethical committee, informed written consent was obtained from 120 women attending the infertility outpatient clinic.

This study was conducted on women with PCO (based on Rotterdam criteria, ESHRE/ASRM 2004), the diagnosis of PCOS is determined by the presence of two of the following conditions: oligo-ovulation or anovulation, hyperandrogenism and polycystic ovaries detected by ultrasonography with the presence of 12 or more follicles measuring 2–9 mm in diameter, and/or at least one enlarged ovary (>10 cm^3) [7]. None of the participants had history of CC resistance.

Exclusion criteria included women with endocrinological abnormalities as thyroid dysfunction or abnormal prolactin

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levels, those with hypothalamic or pituitary dysfunctions evaluated by low gonadotropin level, other causes of infertility as tubal factor evaluated by HSG or laparoscopy, abnormal uterine cavity evaluated by sonohystrography or hysteroscopy and male factor evaluated by semen analysis. Women with ovarian cysts and those with allergy to used medications were also excluded from the study. Patients who had received any hormonal medications (except progesterone for withdrawal bleeding) within the last three months before the study were also excluded.

The patients were subjected to history taking, including age, duration and type of infertility and medical history. Full examination, including general and abdominal and vaginal examination, was done followed by ultrasound evaluation for exclusion of ovarian cysts. Hirsutism was diagnosed with a Feriman Gallway Score of $\geq$8. Basal Hormonal assessment (day 3 FSH, LH, E2 and prolactin) was done to ensure adherence to inclusion criteria.

Patients were randomized at the beginning of each cycle by sealed opaque envelopes containing random generated numbers into three groups. All patients received CC (clomid global Napi, 6th October Egypt) 100 mg orally in two divided doses from day 3 until day 7 of the menstrual cycle. Group I (40 patients) received no further treatment. Group II (40 patients) received 1200 mg NAC (acetylcysteine, SEDICO CO., Egypt) in two divided doses in the form of powder inserted in small pockets to be diluted into one standard glass of water from day 3 until day 7 of the menstrual cycle. Group 3 (40 patients) received metformin 500 mg (cidophage 500 mg, CID CO., Egypt) three times daily continuously.

A transvaginal ultrasonography using Toshiba femio 5 (Toshiba Medical Solutions Inc., Ultrasound Division, Japan) equipped with a 6.5 MHz transvaginal transducer was done on alternate days starting from cycle day 9 till ovulation to determine the follicular number, size, endometrial thickness and pattern and evidence of follicular rupture.

Successful ovulation occurs when the presence of at least one follicle with more than 18–20 mm in size. Peak endometrial thickness was measured at that follicular size. HCG 10 000 IU was given intramuscular when the leading follicle reaches 18–20 mm and timed intercourse was advised.

Treatment was repeated in non-pregnant cases for three successive cycles.

The primary outcome parameter evaluated was spontaneous clinical pregnancy (defined as the presence of gestational sac containing fetal hearts on ultrasound scan). Other parameters included occurrence and day of ovulation, peak endometrial thickness and pattern, number and size of follicles.

Statistical analysis

All collected questionnaires were revised for completeness and consistency. Pre-coded data were entered on the computer using ''Microsoft Office Excel Software'' program (2010) (Redmond, WA) for windows. Data were then transferred to the Statistical Package of Social Science Software program, version 12 (SPSS Inc., Chicago, IL) to be statistically analyzed.

Data were summarized using mean and standard deviation for quantitative variables and frequency and percentage for qualitative ones.

Comparison between groups was performed using independent sample *t*-test or one-way analysis of variance with *post hoc* Tukey's test for quantitative variables and chi square or Fissure exact test for qualitative ones p values less than 0.05 were considered statistically significant, and less than 0.01 were considered highly significant.

Results

One hundred and twenty women with PCOS included in this study were randomly divided to 3 groups: group I included 40 women received CC only, group II included 40 women received CC plus NAC and group III included 40 women received CC plus metformin.

There was no significant difference between the three study groups regarding age, body mass index (BMI), duration of infertility, type of infertility and menstrual pattern (Table 1).

There was no significant difference between the three study groups regarding Basal day 3 FSH, LH, serum estradiol, mean LH/FSH ratio and number of cases with hirsutism and those with ultrasonographic picture of PCO (Table 1).

There was no significant difference between the three study groups regarding number of intermediate follicles 14–18 mm but a significantly higher number of small follicles <14 mm in group II when compared with the other two groups (Table 2).

There was a statistically significant difference between group II and other two groups regarding average number of follicles <18 mm per cycle (Table 2).

There was no significant difference between the three study groups regarding occurrence and laterality of ovulation and pregnancy rate per cycle (Table 2).

There was a highly statistically significant difference between group II and the other two groups regarding peak endometrial thickness (Table 2).

There was a statistically significant difference between group II and other two groups regarding pregnancy rate per patient (Table 2).

There were only minor adverse effects as 1 woman in GI experienced nausea and 1 woman in GIII had drowsiness.

Discussion

Adding NAC to CC has increased number of dominant follicles and improved endometrial thickness with improvement of pregnancy rates per patient but not pregnancy rate per cycle per cycle.

In our study, women received NAC had more dominant follicles than women received CC only or CC and metformin (2.25 ± 0.62 versus 1.75 ± 0.7 and 1.89 ± 0.6, respectively) (p value 0.02 and 0.04, respectively).

In our study, we found that women received NAC had thicker endometrium than women received CC only or CC and metformin (6.7 ± 1.1 versus 5.4 ± 0.6 and 5.3 ± 0.6, respectively) (p value <0.001).

NAC administration increases ovulation rate and eliminate negative impact of CC on endometrial thickness through antioxidant effects and the potential insulin-sensitizing effects of NAC may lead to better induction of ovulation in these patients [8].

Most of the beneficial effects of orally administered NAC are theorized to be due to its ability to either reduce extracellular cystine to cysteine or to be a source of sulfhydryl metabolites. As a source of sulfhydryl groups, NAC can stimulate glutathione synthesis, enhance glutathione-*S* transferase activity, promote detoxification, and act directly on reactive oxidant radicals [9].

In our study, the addition of metformin to CC did not improve ovulation rate (45% versus 40%), endometrial thickness (5.3 ± 0.6 versus 5.4 ± 0.6 mm) and pregnancy rate per cycle (22.2 versus 25%).

Palomba et al. (2005) found that women with PCOS who received metformin had a nearly similar ovulation rate (63 versus 67%) but superior pregnancy rates (69% versus 34%) compared with those received CC only. However, their study had a weak point of including only women with normal-weight and most women with PCOS are overweight [10].

Table 1. Characteristics among study groups.

				p Value		
	GI	GII	GIII	I*II	I*III	II*III
Age (years)[a]	26.0 ± 3.6	25.8 ± 3.5	25.8 ± 3.5	1.0	1.0	1.0
BMI (kg/m^2)[a]	27.3 ± 3.2	27.4 ± 3.1	27.7 ± 2.9	1.0	0.9	0.9
Duration of infertility (years)[a]	2.8 ± 0.8	2.8 ± 0.8	2.7 ± 0.7	1.0	0.8	0.9
Type of infertility[b]				1.0	0.7	1.0
Primary	26 (65.0%)	24 (60.0%)	22 (55.0%)			
Secondary	14 (35.0%)	16 (40.0%)	18 (45.0%)			
Menstrual pattern[b]				0.6	0.7	0.9
Regular	10 (25.0%)	16 (40.0%)	14 (35.0%)			
Oligo	26 (65.0%)	22 (55.0%)	24 (60.0%)			
Ameno	4 (10.0%)	2 (5.0%)	2 (5.0%)			
Hirsutism[b]				0.7	0.7	1.0
Hirsute	26 (65.0%)	22 (55.0%)	20 (50.0%)			
None	14 (35.0%)	18 (45.0%)	20 (50.0%)			
FGS score[c]	7.3 ± 1.8	6.9 ± 1.9	6.4 ± 1.1	0.6	0.4	0.3
Basal FSH (mIU/ml)	4.23 ± 1.1	4.46 ± 0.9	3.99 ± 1.1	0.8	0.6	0.6
Basal LH (mIU/ml)	8.65 ± 1.88	9.1 ± 1.9	8.76 ± 1.9	0.7	0.9	0.6
Estrdiol (pg/ml)	67.23 ± 53.8	62.35 ± 54.9	71.8 ± 61.6	0.6	0.5	0.3
LH/FSH ratio[a]	1.4 ± 0.3	1.5 ± 0.2	1.5 ± 0.2	0.7	1.0	0.8
US picture of PCO[b]				1.0	1.0	1.0
Yes	32 (80.0%)	34 (85.0%)	34 (85.0%)			
No	8 (20.0%)	6 (15.0%)	6 (15.0%)			

[a]Data are presented as mean ± SD.
[b]Data are presented as number (percent).
[c]FGS Feriman Gallway Score.

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Table 2. Outcome parameters among study groups.

				p Value		
	GI	GII	GIII	I*II	I*III	II*III
Follicles >14 mm[a]	22 (55%)	10 (25%)	20 (50%)	**0.04 S**	1.0	**0.05 S**
Follicles 14–18 mm[a]	4 (10%)	10 (25%)	4 (10%)	0.4	1.0	0.4
Average number of follicles <18 mm per cycle[b]	1.75 ± 0.7	2.25 ± 0.62	1.89 ± 0.6	**0.02 S**	0.5	**0.04 S**
Ovulation[a]				0.3	1.0	0.5
Yes	16 (40.0%)	24 (60.0%)	18 (45.0%)			
No	24 (60.0%)	16 (40.0%)	22 (55.0%)			
Laterality of ovulation[a]				0.6	1.0	0.3
Unilateral	14 (87.5%)	16 (66.7%)	16 (88.9%)			
Bilateral	2 (12.5%)	8 (33.3%)	2 (11.1%)			
Endometrial thickness[b] (mm)	5.4 ± 0.6	7.3 ± 1.1	5.3 ± 0.6	**<0.001**	1.0	**<0.001**
Pregnancy rate per cycle[a]				1.0	1.0	0.7
Yes	4 (25.0%)	8 (33.3.0%)	4 (22.2%)			
No	12 (75.0%)	16 (66.7%)	14 (77.8%)			
Pregnancy rate per patient[a]				**0.05 S**	1.0	**0.05 S**
Yes	4 (10.0%)	8 (20.0%)	4 (10.0%)			
No	36 (90.0%)	32 (80.0%)	36 (90.0%)			

Bold values indicate statistically significant.
[a]Data are presented as number (percent).
[b]Data are presented as mean ± SD.

Moghetti et al. (2000) demonstrated that long-term efficacy of metformin treatment in obese women with PCOS. The study involved 32 obese women with PCOS (average BMI 30 kg/m^2) who were treated with either placebo or 1500 mg metformin daily for 6 months. The findings demonstrated marked improvements in menstrual cyclicity and ovulation with significant reduction in serum androgens in the treated versus placebo arm. However, small sample size is still a weakness [11].

Nestler et al. (1998) demonstrated that improved menstrual cyclicity in PCOS. They treated 61 moderately obese women with PCOS for 5 weeks with either metformin or placebo and then given CC (50 mg for 5 days) to induce ovulation. Metformin therapy resulted in an 8-fold increase in spontaneous ovulation during the pretreatment period and a greater than 10-fold increase in CC-induced ovulation (2 of the 25 women 8% who received placebo plus CC ovulated ($p < 0.001$). Overall, 31 of the 35 women 89% treated with metformin ovulated spontaneously or in response to CC, as compared with 3 of the 26 women 12% treated with placebo) [4]. That confirms the role of metformin in reversing hormonal imbalance found in PCOS women.

Saghar et al. (2012) noted that the rate of ovulation was 45.12% in the CC + NAC group versus 28% in the CC only group with better endometrial thickness in the CC + NAC group 6.6 ± 1.69 mm versus 5.4 ± 1.61 mm in the CC only group and higher pregnancy rates 20.73 in the CC + NAC group versus 9.4%

in the CC only group [12]. This results add a confirmatory evidence to our study.

Rizk et al. (2005) 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 (49.3% in CC + NAC group compared with 1.3% in the placebo group) and pregnancy rate (21.3% in CC + NAC group compared with 0% in the placebo group) but did not reveal any significant change in endometrial thickness [13].

Badawy et al. (2007) noted that compared with placebo, the addition of NAC to a CC regimen in patients with PCOS increased ovulation rates significantly (52.1% in CC + NAC group compared with 17.9% in the CC only group), pregnancy rate (11.5% in CC + NAC group compared with 0% in the CC only group) and endometrial thickness (7.3 ± 3.1 mm in CC + NAC group compared with 4.3 ± 1.2 mm in the CC only group) [8].

A systematic review done to evaluate the benefits and harms of NAC in women with PCOS. Eight studies with a total of 910 women with PCOS were randomized to NAC or other treatments/placebo. There were high risk of selection, performance and attrition bias in two studies and high risk of reporting bias in four studies. Women with NAC had higher odds of having a live birth, getting pregnant and ovulation as compared with placebo. However, women with NAC were less likely to have pregnancy or ovulation as compared with metformin. There was no significant difference in rates of the miscarriage, menstrual regulation, acne, hirsutism, and adverse events, or change in BMI, testosterone, and insulin levels with NAC as compared with placebo. It concluded that NAC showed significant improvement in pregnancy and ovulation rate as compared with placebo [14].

We concluded that NAC as an adjuvant to CC for induction of ovulation improved the ovulation and pregnancy rates in PCOS patients and have 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.

Finally, Ovulation rates were higher among PCOS patients receiving NAC as an adjuvant to CC for induction of ovulation more than those receiving metformin as an adjuvant to CC for induction of ovulation with significant improvement of endometrial thickness.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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