N-acetyl cysteine in ovulation induction of PCOS women underwent intrauterine insemination: An RCT

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Abstract

Background: N-acetyl cysteine (NAC) was proposed as an adjuvant to clomiphene citrate for ovulation induction in patients with polycystic ovary syndrome (PCOS) without clomiphene citrate resistance.

Objective: To evaluate the effect of NAC on pregnancy rate in PCOS patients who were candidates for intrauterine insemination.

Materials and Methods: In this randomized clinical trial 97 PCOS women aged 18-38 years were enrolled in two groups, randomly. For the case group (n=49), NAC (1.2 gr)+ clomiphene citrate (100 mg) + letrozole (5mg) were prescribed Daily from the third day of menstruation cycle for five days. The control group (n=48) had the same drug regimen without NAC. In order to follicular development, GONALF was injected on days of 7-11 menstrual cycles in all participants. When the follicle size was 18mm or more, HCG (10000 IU) was injected intramuscular and the intrauterine insemination was performed after 34-36 hr.

Results: There were not significant differences between study groups regarding mean endometrial thickness (p=0.14), mean number of mature follicles (p=0.20) and the pregnancy rate (p=0.09).

Conclusion: NAC is ineffective in inducing or augmenting ovulation in PCOS patients who were candidates for intrauterine insemination and cannot be recommended as an adjuvant to CC in such patients.

Keywords: N-acetyl cysteine, Polycystic ovary syndrome, Intrauterine insemination, Ovulation induction.

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Introduction

Polycystic ovary syndrome (PCOS) is the most common cause of anovulation among reproductive-age women (1, 2). It is characterized by hyperandrogenism, insulin resistance, and chronic anovulation and affects 5-10% of women in reproductive age (3-5).

Clomiphene citrate (CC) is recommended as the first treatment strategy to induce ovulation in these patients (6). Patients who do not ovulate while receiving even 150 mg CC are classified as CC-resistant patients (7). In these cases, other treatment regimens are recommended such as the co-administration of metformin and CC (that its impact is questioned), Gonadotropin (which has high costs and side effects such as multiple pregnancies and ovarian hyperstimulation syndrome), and ovarian drilling by diathermy (8, 9). However, a recent Cochrane review revealed that whereas metformin was associated with improved clinical pregnancy and ovulation rate, it did not improve live birth rates when used alone or in combination with CC or when compared with CC (10). Therefore, there is a need for developing therapeutic selections for treating the women with PCOS.

Aromatase is a cytochrome P450 enzyme which converts androstenedione and testosterone to estrone and estradiol by hydroxylation (11). Aromatase Inhibitors particularly letrozole have been prescribed for ovulation induction in patients who do not respond to CC (12). With respect to the performance of aromatase, the enzyme inhibitor medicines inhibit the estrogen production and consequently, the pituitary gland is stimulated to secrete FSH and ovarian follicle grows larger followed by FSH (13).

N-Acetyl Cysteine (NAC) with the chemical structure of sulfhydryl groups is derived from L- amino acid cysteine and the first time were
used as a mucolytic drug in some respiratory
diseases such as chronic bronchitis (14). NAC
decreased blood levels of homocysteine by
increasing glutathione synthesis which is an
antioxidant (15). On the other hand, NAC
significantly reduces the serum testosterone
level, insulin resistance, and serum lipids (16).
It was shown that the combination of NAC and
CC enhance ovulation rate and pregnancy
rate in CC-resistant PCOS patients (17). By
administering NAC with CC in PCOS patients
without CC resistance, a considerable
improvement in the ovulation rate, serum
estrogen and progesterone, endometrial
thickness, and pregnancy rate were observed
(15, 18). According to the results of Salehpour
study, NAC is as a safe and well-tolerated
adjuvant to CC for induction of ovulation can
improve the ovulation and pregnancy rates in
PCOS patients (19).

We aimed to evaluate the effect of NAC
with a combination of two inductions of
ovulation drugs in PCOS patients who were
candidates for intrauterine insemination (IUI),
to assess the pregnancy rate.

Materials and methods

This randomized clinical trial was
performed on 100 PCOS women candidated
intrauterine insemination (IUI) who referred to
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Gynecology, Shahid Motahhari Hospital,
Urmia University of Medical Sciences, Iran.
After obtaining the informed constant, the
participants were randomized using closed
envelopes (A and B) in two groups: The case
group (received NAC) and control group
(without NAC). Inclusion criteria were PCOS
women who were IUI candidate, age 18-38
years, having two out of three criteria of
chronic oligo- or anovulation, clinical or
laboratory signs of hyperandrogenism, PCOS
sonographic findings (based on the Rotterdam
criteria (20) also the presence of normal
laboratory tests of thyroid, prolactin, and
normal hysterosalpingography and a normal
transvaginal ultrasound. Exclusion criteria
included, the presence of an ovarian cyst,
FSH> 10 IU/L, and patients with ovarian hyper
stimulation syndrome (OHSS) and male
infertility.

The sample size was calculated based on
a level error of 5% and a power of 80% to see
a difference between two groups. The
estimated sample size was 84 patients (42
patients in each group), but for reaffirmation
100 patients were selected by simple random
method. Three patients in case group and six
patients in control because of discontinuation
of treatments did not receive allocated
intervention.

The case group were received 100 mg CC
(Clomid©, Hoechest Marion Russel, Cairo,
Egypt) and 5 mg letrozole (Novartis Pharma
Services, Basel, Switzerland) plus 1.2 gr NAC
(Sedico, Cairo, ARE) daily, from day 3 to 7 of
the menstrual cycle for one 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.

Then 75 IU of GONAL-F (Gonal-F, Merck,
Serono, Germany) was injected
subcutaneously from the seventh of menstrual
cycles and continued to 11th of the cycle. On
the twelfth 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, 10000 IU hCG (Profasi. Serono.
Switzerland) was injected intramuscularly and
36-40 hr after hCG injection, IUI was
performed. The serum $\beta$-hCG level was
measured on the sixteenth day after hCG
injection. In the control group, the process
was same as above, but without the NAC.

Finally, the treatment duration until the
presence of mature follicle, number and size
of mature follicles, endometrial thickness, the
timing of HCG injection, and pregnancy,
miscarriage, multiple pregnancies, and ectopic
pregnancy were compared in two
groups. Meanwhile, transvaginal ultrasound
was performed using 7.3 MHz Probe/Fukuda
Denshi. Clinical pregnancy was defined as the
presence of a gestational sac on ultrasound,
as confirmed by the presence of a fetal heart
rate. Secondary outcomes were related to
assessing of abortion, OHSS, and multiple
pregnancies.

Ethical Considerations

This study was approved by the ethics
committee of Urmia University of Medical
Sciences (Ir.UMSU.rec.1393.179) Subjects
were informed that their participation was
voluntary and written consent was obtained
from all participants.
Statistical analysis
For continuous variables, data were presented as means±SD and for categorical variables, as the number and frequency. The comparisons of variables between the two groups were made using the Fisher’s exact test for categorical variables and independent samples t-test for continuous variables. Kolmogorov-Smirnov test for normality quantitative data was reviewed. T-test was used for normally distributed data. Statistical analysis was performed using SPSS Inc, Chicago, Illinois, USA (SPSS) version 17. All the cut-off for statistical significance presumed 0.05.

Results
Totally, there were 106 patients in both groups at the start of this study but three of whom from the case group and 6 women from controls were excluded due to the discontinuation of intervention. Therefore, 97 patients continued our study: Case group (n=49) and control group (n=48) (Figure 1).

Table I shows Demographic characteristic of study participants. There were no statistically significant differences in age, body mass index, duration of infertility, the mean of LH and FSH, type of infertility, the mean of mature follicles, and mean number of Gonal-F in two groups (Table I, II).

The mean number of mature follicles was 2.10±0.87 in the case group and 1.85±1.03 in the control group. There was no significant difference between two groups regarding the mean number of mature follicles (p=0.20), the mean endometrial thickness on the day of HCG administration by transvaginal sonography (p=0.25), the mean day of HCG administration (p=0.47), and the mean day of IUI performing (p=0.63) (Table II).

In the case group (n=49) with 49 patients, pregnancy test was positive in 16 patients (32.7%), but pregnancy test was negative in 33 patients (67.3%). Also, in the control group with 48 patients; pregnancy test was positive for 9 (18.8%) women and negative among 39 (81.2%) women. According to Fisher test, there was no significant difference between two groups regarding pregnancy rate (p=0.09). Besides, one case of abortion, one ectopic pregnancy (EP), and one twin pregnancy have been reported in the experimental group (Table II). No manifestations of ovarian hyper stimulation syndrome (OHSS) were reported in two groups.

| Table I. Demographic characteristic of study participants |
|----------------------------------------------------------|
| Variables                                               | Case group (n=49) | Control group (n=48) | p-value |
| Age (years)*                                            | 27.53±4.16        | 27.14±4.49           | 0.64    |
| Body mass index (kg/m²)*                                | 27.23±2.90        | 27.84±2.89           | 0.28    |
| Duration of infertility(year)*                          | 4.26±3.27         | 3.40±2.20            | 0.17    |
| Serum FSH level (mIU/mL) *                              | 6.51±1.89         | 6.68±2               | 0.66    |
| Type of infertility n (%)                               | 39 (75%)          | 43 (79.6%)           | 0.36    |
| Primary                                                |                  |                      |         |
| Secondary                                              | 13 (25%)          | 11 (20.4%)           |         |

* Data presented as Mean±SD
T-Test, Chi-square Test

| Table II. Assessed Quantity variable after intervention among two group |
|------------------------------------------------------------------------|
| Variables                                                              | Case group (n=49) | Control group (n=48) | p-value |
| No. of mature follicles*                                               | 2.10±0.87         | 4.33±2.27            | 0.83    |
| No. of GONAL-F injection*                                              | 4.25±1.72         | 8.04±1.4             | 0.25    |
| Endometrial thickness (mm)*                                            | 8.15±0.85         | 14.29±1.82           | 0.47    |
| Day of HCG administration*                                             | 14.04±1.84        | 14.87±2.68           | 0.63    |
| Day of IUI performing*                                                 | 15.93±1.74        | 9(18.8%)             | 0.69    |
| Pregnancy rate**                                                      | 16(32.7%)         | 9(18.8%)             | 0.69    |
| Miscarriage rate**                                                    | 16(32.7%)         | 0(0.0%)              | 0.62    |
| EP rate**                                                             | 16(32.7%)         | 0(0.0%)              | 0.62    |
| Twin pregnancy **                                                     | 16(32.7%)         | 0(0.0%)              | 0.62    |

* Data presented as Mean±SD
** Data presented as n (%)
EP= Ectopic pregnancy
*T-Test, Fisher Test
Discussion

To the best of our knowledge, this is the first published study comparing the effect of NAC in PCOS women who were candidates for IUI. The metaanalysis was conducted to assess clinical benefits of NAC among women with PCOS. A total of eight randomized controlled trials with 910 women compared the effects of NAC with placebo or metformin in women with PCOS. NAC significantly improved rates of live birth and spontaneous ovulation compared to placebo in women with PCOS (21). However, we found no evidence of effects of NAC on improving pregnancy rate and spontaneous ovulations.

Also, in the present study the endometrial thickness on the day of HCG administration and pregnancy rate were not significant difference in two groups. While in the study from Salehpour et al, the number of follicles larger than 18 mm and the mean of endometrial thickness in the group who received CC and NAC (for six weeks) were significantly higher than those who only received CC. As well as the rate of ovulation and pregnancy in the study group who received CC and NAC was significantly greater than in the group receiving CC. Finally, they concluded that adding NAC to CC as a non-harmful adjuvant improve the ovulation rate and increases pregnancy rates (19).

Like Salehpour et al study, Rizk et al have stated in patients who received NAC in combination with CC, ovulation and pregnancy rates were significantly increased and no cases of OHSS has been reported (22). Nasr studied the outcomes of NAC administration on CC-resistant PCOS patients who underwent unilateral laparoscopic ovarian drilling. In his study, the group receiving NAC had a significantly high rate of ovulation, pregnancy and live birth rate while the abortion rate was significantly low (22). Thakker et al showed that the pregnancy, ovulation and live birth rates were high in the received NAC group compared with the placebo group (21). Millea study stated that NAC administration with a dose of 1200 mg improves ovulation and pregnancy rates in PCOS patients which can be due to a decrease in insulin resistance (24).

Oner et al performed a comparison study on the effects of metformin and NAC in PCOS patients, they concluded that both NAC and metformin leads to decrease in BMI, hirsutism, menstrual irregularities, levels of fasting insulin and free testosterone (25). Amin et al had a study on repeated failure of pregnancy and came to the conclusion that the concurrent use of NAC with folic acid compared to taking folic acid alone, allow to progress further 20 wk of pregnancy and significantly increase live birth rate (26). AbuHashim et al concluded that co-administration of metformin and CC had better results compared with NAC and CC in increasing rates of ovulation and pregnancy and even in reducing the abortion rate. As well as in patients receiving metformin and CC, estrogen and progesterone levels and also endometrial thickness were at a high level on the day of HCG administration (27).
A prospective randomized placebo-controlled pilot study on 60 Iranian women with PCOS (aged 25-35 yr) undergoing intracytoplasmic sperm injection showed that NAC (1800 mg) improves oocyte and embryo quality and could be administered as an alternative to Metformin (28) Also, NAC as an adjuvant to CC for induction of ovulation improves ovulation and pregnancy rates in PCOS patients with beneficial impacts on endometrial thickness (29). A systematic review of clinical trials showed that antioxidants and vitamins have positive effects on the management of PCOS women. Although it seems more studies is necessary for this field (30).

Although the results of our study and the above studies were different we can conclude that administration of NAC in CC-resistant PCOS patients, has no effect on follicles maturation, and endometrial thickness. Some studies have suggested that reduced insulin resistance is as a mechanism for NAC performance. On the other hand, since metformin mechanism is similar with NAC, it seems that different results on the combined use of NAC with these two drugs CC and letrozole are affected by other factors such as genetic factors, dosage and duration of drug use and the sample size.

So, further studies are needed to be done on NAC therapy effects with a larger sampling in the large geographical areas and assessment of NAC therapy effects on biomedical, hormonal and metabolic profiles, symptoms of hyperandrogenism, and cardiovascular risk factors. However, our study was limited to one treatment cycle, raising the dosage of NAC will further increase the cost, which is an important consideration in choosing the appropriate therapy.

**Conclusion**

NAC is not effective in inducing or augmenting ovulation in PCOS patients who were candidates for IUI and cannot be recommended as an adjuvant to CC in such patients.

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**Conflict of interest**

It should be noted that there was no association between the authors and any organization or institution. The Authors report no declarations of interest.

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