

## The effectiveness of using n-acetyl cysteine together with clomiphene citrate on ovulation induction in the patients with polycystic ovary syndrome referred to Isfahan reproduction and infertility center in 2013

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**Abstract:** A common cause of infertility is unovulation that different medications and various methods are used to treat it. Clomiphene Citrate (Clomid) and N-acetyl Cysteine (NAC) are among ovulation medications. In this study, the effect of Clomiphene Citrate alone and in combination with N-acetyl Cysteine was compared in the treatment of infertility. In this randomized clinical trial, two groups of 36 subjects were selected from the PCOs referred to Isfahan Infertility Center. In the first group, from the third day of menstruation 100 mg Clomiphene Citrate tablets were prescribed to be taken one per day and for five days. On the eleventh day of menstrual these subjects were examined in terms of follicles' number and size. 10,000 units of HCG were injected and the results were evaluated in terms of the number and size of follicles. The subjects of the second group received 600 mg oral N-acetyl Cysteine for 2 months and three times a day, and then, like the first group, were treated by clomiphene. The age of the subjects was between 18 and 35 (with the mean age of 27.97). The mean duration of infertility has been 2.96 years. The average number of follicles was similar in two groups before taking and medication. The mean number of follicles in the second group (after taking Clomiphene Citrate together with N-acetyl Cysteine) was more than the first group (Clomiphene Citrate). Moreover, the mean increase in the number ovarian follicles was more in the second group than the first one. The subjects of the second group, in a significant difference from the subjects of the first group, found two follicles. According to the results of this study, using N-acetyl Cysteine together with Clomiphene Citrate will lead to better ovulation in infertile women.

**Key words:** Clomiphene Citrate; N-acetyl Cysteine; Ovulation; Polycystic ovary syndrome; Infertility

### 1. Introduction

Infertility is defined as the state of nulliparity within one year of unprotected coition (Berek et al., 2012). One of the main causes of chronic anovulation in the women of reproductive age is polycystic ovary syndrome (Ciotta et al., 2011). This syndrome includes almost 6-10% of women in reproductive age (Enrico papaleo et al., 2008). The pathogenesis of polycystic ovary syndrome is unknown; however, TGF<sub>1</sub> activity on solo ovary cells and peripheral tissues causes a number of clinical and biochemical features such as hyperandrogenism, insulin resistance, increased secretion of adrenal and chronic anovulation (Kazer, 1989). Polycystic ovary syndrome shows itself with irregularities of menstrual cycle, metabolic disorders, hormonal disorders, chronic anovulation, high levels of androgens, hyper-amenorrhea androgenemia, and symptoms identified in sonography. In 1980, Burghen showed that polycystic ovary syndrome is strongly associated with hyperinsulinemia (Enrico papaleo et al., 2008). Hyperinsulinemia is associated

with an increased risk of cardio vascular diseases, increased risk of diabetes, and several metabolic disorders. This relationship has led to the treatment of women with polycystic ovary syndrome by insulin receptor agonists such as Troglitazone, and N-acetyl Cysteine and metformin for spontaneous ovulation (Tang et al., 2012). N-acetyl Cysteine and metformin have significant effects on hyperandrogenism, hyperinsulinemia and menstrual irregularities in the women with polycystic ovary syndrome (Le Donne et al., 2012).

In the treatment of the women with polycystic ovary syndrome, Clomiphene Citrate is prescribed as a partial agonist of estrogen that is taken orally, well absorbed and lives for 5-7 days. Clomiphene inhibits the inhibitory effects of estrogen on hypothalamus, increases the secretion of Gonadotropin and finally leads to ovulation (Fritz and Sperof, 2012).

Conventional treatment of PCOs is to make ovary to ovulate which uses Clomiphene Citrate as the first-line medicine. Given the clear relationship between the increased insulin levels and insulin resistance, the use of insulin sensitizers such as metformin in ovulation induction has been recently studied that has been successful. Recently a research

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has proven that 34% of the women who were treated with metformin – while they have not used and ovulation induction medicine – have had spontaneous ovulation. While this number has been 4% in the women who had used placebo. 90% of the women who failed to ovulate after taking metformin, had ovulation after using 50 mg Clomiphene Citrate, that this number was 8% in the group who used placebo. Recently, owing to the increased prevalence of polycystic ovary syndrome in Iran and the lack of extensive studies on antioxidants, we decided to, through investigating the effect of N-acetyl Cysteine, evaluate its impact on the number and size of follicles. The purpose of the current study is to evaluate the effect of oral N-acetyl Cysteine, as an antioxidant, together with Clomiphene Citrate on improving the size and number of follicles, increasing fertility and improving the sensitivity of receptors to insulin in a number of women with polycystic ovary syndrome who have referred to Isfahan Infertility Center. In this study, the effect of this medication as the ovulation induction medicine on infertile women is evaluated. Also, during the study, this medicine may improve the metabolic disorder.

## 2. Materials and methods

This study is a randomized clinical trial conducted on the patients referred to Isfahan Infertility Center. In this study, such information as first and last names, age (ranged between 19-40), duration of fertility (between 3-5 years), sonography results and such tests as TSH, PRL, E<sub>2</sub>, FSH, LH, insulin and OGT were collected by the attached questionnaire.

Inclusion criteria include: patients aged less than 40 years, women with PCOS who have 2 criteria from the 3 criteria of Rotterdam (1. Unovulation; 2. Hyperandrogenemia, hirsutism and acne; 3. Observing polycystic ovary in sonography), were selected as the patients with polycystic ovary syndrome.

Exclusion criteria: infertile women with causes other than polycystic ovary syndrome, subjects with hyperinsulinemia, hyperprolactinemia, thyroid dysfunction and a severe increase in androgen (adrenal hyperplasia, Cushing syndrome).

Selected women with polycystic ovary syndrome were randomly divided into two groups that each group included 36 women. In the first group, an ineffective pill (placebo) was prescribed for two months. Then, on the third day of menstruation 100 mg Clomiphene Citrate tablets were given to be used daily for five days. On the eleventh day of menstruation, these women were examined in terms of follicle's size and number (the appearance of one follicle with the diameter of 18-20 mm was considered satisfactory). After this stage, 10,000 units of HCG (pregnyl) were injected intramuscularly. Finally, the results were evaluated in terms of ovarian response (the number and size of grown follicles), pregnancy (positive BHCG > 10), and

gestational sac observed on sonography one week after positivity of BHCG test.

In the second group, 600 mg oral N-acetyl Cysteine was given for two months and three times a day and after these two months the same measures of the first group were performed in this group. Sonography was performed by an obstetrician who was familiar with vaginal and ovarian sonography. Sonography was done using MEDISON SONACE × KOREA and vaginal probe machine. The study's data were analyzed using SPSS software version 20, and, descriptive results by using tables and charts and analytical results were obtained through using independent t-test.

## 3. Results

In this study, 72 women with polycystic ovary syndrome were selected and were divided into two groups each of them included 36 women. The first group received Clomiphene Citrate with placebo and the second group received Clomiphene Citrate together with N-acetyl Cysteine. The age range of the subjects was between 18 and 38 with the mean of  $27.97 \pm 4.8$ . The average age of the first and the second group was respectively  $28.44 \pm 5.35$  and  $27.5 \pm 4.21$ , and according to t-test, no significant difference was observed between two groups ( $P=0.41$ ).

The mean duration of infertility in the two groups was respectively  $2.81 \pm 1.47$  and  $3.11 \pm 2.36$  years, and according to the mentioned test, no significant difference was observed between two groups ( $P=0.51$ ). The average number of ovarian follicles before taking the medicine was 1.00 in the first group and 1.08 in the second group, and based on t-test, no significant difference was observed between two groups ( $P=0.083$ ). The average number of ovarian follicles after taking the medicine was 1.33 in the first group (the group of Clomiphene Citrate with placebo) and 1.69 in the second group (the group of Clomiphene Citrate with N-acetyl Cysteine), and according to t-test there was a significant difference between two groups ( $P=0.002$ ). Likewise, the mean increase in the number of ovarian follicles after taking the medicine was 0.33 in the first and 0.64 in the second group that the difference was significant between two groups ( $P=0.009$ ).

Before treatment, all women of the first group had only one follicle and three women of the second group (8.3%) had two ovarian follicles, and according to Chi-square test, no significant difference was observed between two groups ( $P=0.08$ ). After treatment, 12 subjects of the first group (about 33.3%) and 25 subjects of the second group (69.4%) had two ovarian follicles, that according to Chi-square test, there was a significant difference between the two groups ( $P=0.002$ ). (Table 1):

The mean and standard of ovarian follicles size both before and after treatment for both groups are given in table 2, and according to the above test, no significant difference was observed between two groups ( $P=0.44$ ).

**Table 1:** The frequency distribution of the number of the ovarian follicles before and after the treatment in the control and intervention groups

Groups	The number of follicles before the treatment		The number of follicles after the treatment		Total
	1	2	1	2	
First	36(100%)	0	24(66.7%)	12(33.3%)	36(100%)
Second	33(91.7%)	3(8.3%)	11(30.6%)	25(69.4%)	36(100%)

Regarding the size of the second follicle, in the first group no one had two follicles before receiving the medicine, and the average size of the second ovarian follicles, before taking the medicine, was  $15.25 \pm 3.12$  in the second group. However, after

taking the medicine, the average size of the second follicle was  $15.25 \pm 3.12$  in the first group and  $16.64 \pm 3.85$  in the second group, and according to the t-test no significant difference was observed between two groups ( $P=0.28$ ).

**Table 2:** The distribution of the ovarian follicle average size both before and after the treatment in both groups

Groups	The average size of follicles before the treatment	The average size of follicles after the treatment	The mean size of the follicles before and after the treatment
First	$13.60 \pm 1.84$	$14.40 \pm 3.96$	$0.81 \pm 3.99$
Second	$12.92 \pm 2.37$	$14.47 \pm 3.74$	$1.56 \pm 4.14$
P-Value	0.18	0.94	0.44

#### 4. Discussion and Conclusion:

The difference between the average age of the two groups was not statistically and at the level of 0.05 significant, and in terms of age, the two groups were somehow similar. The study conducted by Safdarian and Motahari (2013) showed that ovulation is significantly related with age and the duration of infertility. Pregnancy also had a statistically significant correlation with age and the duration of infertility

The subjects' duration of infertility has been between 1 and 12 years with the average of  $2.96 \pm 1.96$ . The average duration of infertility was 2.81 years in the first group and 3.11 years in the second group. The mean duration of infertility between the two groups was not statistically significant and both groups were almost similar in terms of infertility duration.

According to Rahmani et al. (2012) there was no significant difference between the group that had received Letrozole and the group that had received Clomiphene in terms of demographic and infertility duration at the outset of the study. Before taking the medicine, two groups were similar in terms of follicles number and no significant difference has been observed between the two groups.

In a study conducted by Lorzadeh et al. (2011) the mean age, body mass index, infertility duration, and ovulation rate, in two groups of Letrozole and Clomiphene Citrate were similar before the treatment. After taking medicine, the two groups were different in terms of ovarian follicles number and the difference was significant, so that the average number of the ovarian follicles in the second group was significantly higher than the first. According to Lorzadeh et al. (2012) the mean number of follicles was  $1.30 \pm 0.50$  in the group treated by Letrozole and  $2.32 \pm 1.58$  in the group treated by Clomiphene Citrate. The mean size of the

follicles and the pregnancy rate were similar in both groups

After the treatment, both groups were significantly different in term of the mean increase in the number of ovarian follicles, so that the number of the follicles has been more increased in the second group than the first group ( $P=0.002$ ).

In a study conducted by Lorzadeh et al. (2012) Endometrial thickness was  $9.71 \pm 1.27$  mm in the group received Letrozole and  $6.08 \pm 3.08$  in the group treated by Clomiphene Citrate. Ovarian rate in both groups was 44 (88%) indicating that there is a significant difference between the two groups before treatment, all subjects of the first group had only one follicle and three subjects of the second group (8.3%) had two follicles, indicating that no significant difference has been between the two groups. After treatment, however, the difference was significant so that much more subjects in the second group had two follicles.

According to the study of Nazari et al. (2011), the low dose of Gonadotropin (HMG) could successfully stimulate follicular response in the women with polycystic ovary syndrome who were resistant to Clomiphene Citrate. Of every 8 women showing follicular response in ovulation induction, two women get pregnant

In the study of Salehpour et al. (2011) in the patients who received Clomiphene Citrate with N-acetyl Cysteine, ovulation rate was significantly higher than the group who received Clomiphene Citrate alone before using medicine, the two groups were similar in terms of follicle size and no significant difference was observed between two groups. After taking the medicine, the difference between the two groups was not statistically significant in terms of follicle size.

The study of Safdarian and Motahari (2013) showed that in the patients resistant to Clomiphene Citrate, using metformin can be effective in inducing ovulation and pregnancy. So that ovulation was observed in 19 women (61.3%) and pregnancy was

seen in 9 women (29%). After taking the medicine, the two groups were not different in terms of increase in follicle size. Although it was observed that the increase was more in the second group than the first one, the difference was not statistically significant which could be due to the low sample size and high variance of the groups.

According to the study of Agha-hosseini et al. (2003), in terms of age, BMI, type of infertility and menstrual, no significant difference was observed in response creation. There was no significant relationship between pregnancy rate and the 21th day progesterone level, and with increasing levels of progesterone the pregnancy rate also increased in both groups (15). The difference between the two groups was not statistically significant in terms of the size of the second follicle and after taking the medicine. However, it was observed that the size of the second follicle was greater in the second group than the first.

In the study of Salehpour et al. (2011) in the patients who have received Clomiphene Citrate with N-acetyl Cysteine, fertility rate and larger than 18mm follicles were significantly higher than the group who have received Clomiphene Citrate alone

In the study of Badawy A. et al. (2007) in the women who have received Clomiphene Citrate with N-acetyl Cysteine, the number of mature follicles, improvement in endometrial thickness, and the increase in fertility rate were significantly higher than the group who have received Clomiphene Citrate alone

The study of Davar and Aflatunian (2003) showed that by adding Clomiphene Citrate or Gonadotropin to Letrozole, its impact on patients will be increased

In the study of Rahmani et al. (2012) follicle creation, the increase of pregnancy rate, abortion and side-effects were not observed in the group received Letrozole and the group received Clomid

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