http://www.ijwhr.net
doi 10.15296/ijwhr.2019.31
Open Access Original Article
IJWHR
International Journal of Women's Health and Reproduction Sciences
Vol. 7, No. 2, April 2019, 185–189
ISSN 2330-4456

Comparison of Pregnancy Rate in Patients With Polycystic Ovary Syndrome Treated With Clomiphene Alone and in Combination With N-acetyl Cysteine: A Randomized Clinical Trial

Nayereh Ghomian[1*], Nayereh Khadem[1], Somayeh Moeindarbari[1], Aghdas Abdolrazagh[1]

Abstract

Objectives: Polycystic ovary syndrome (PCOS) is one of the frequent endocrine disorders among young females, which can cause infertility. Recently, evidence has shown the beneficial impacts of N-acetyl cysteine (NAC) use along with clomiphene citrate on the treatment of infertility caused by PCOS. Regarding this, the present study aimed to compare the pregnancy rate between the PCOS patients treated with clomiphene and those received clomiphene along with NAC.

Materials and Methods: This blinded randomized controlled clinical trial was performed on 66 patients with PCOS who presented with infertility at Milad Infertility Center in Mashhad, Iran, in 2015. The patients were divided into two groups of intervention and control using a random number table. In the intervention group, 100 mg of clomiphene (i.e. two pills) along with 1200 mg of NAC (i.e. two 600 mg pills) were given to the participants from the third day of the menstrual cycle for five days. In the control group, 100 mg of clomiphene was administered from the third day of the cycle for 5 days. The patients' response to medications as well as the number and size of follicles were assessed using the vaginal ultrasound.

Results: The 2 study groups had no significant difference regarding age, duration of infertility, body mass index, history of infertility treatment, and endometrial thickness on the third day of the menstrual cycle. The mean endometrial thickness was 7.47 ± 1.6 (NAC/clomiphene) and 7.58 ± 2.1 mm (clomiphene) on the 12th day of the menstrual cycle ($P = 0.810$). Furthermore, the mean sizes of the follicles were 13.6 ± 4.2 (clomiphene) and 15.9 ± 5.1 mm (NAC/clomiphene) ($P = 0.301$). The mean numbers of follicles were 1.56 ± 0.9 (clomiphene) and 1.8 ± 0.9 (NAC/clomiphene) ($P = 0.069$). In total, 7 (21.2%) and 5 (15.1%) patients in the intervention and control groups had a positive beta-hCG result, respectively ($P = 0.260$).

Conclusions: As the findings of this study revealed, the addition of NAC to clomiphene treatment was not associated with an increased chance of pregnancy in patients with PCOS-related infertility.

Keywords: Clomiphene citrate, Endometrium, N-acetylcysteine, Polycystic ovary syndrome, Infertility

Introduction

Polycystic ovary syndrome (PCOS), also called Stein-Leventhal syndrome, is one of the most common endocrine disorders in young females, the prevalence of which has been reported as 20% using the Rotterdam criteria (1,2). However, the etiology of the disease is unknown. The manifestations of this disease are very diverse, including clinical or laboratory hyperandrogenism, insulin resistance, glucose intolerance, hirsutism, menstrual disorders, anovulation, and oligoovulation.

The patients with PCOS suffer from multiple comorbidities, including infertility, the cause of which is chronic anovulation in 50 to 75% of the infertile females (1,2). The PCOS has a considerable prevalence, which bears physical and mental effects and high treatment cost. Regarding this and given the high prevalence of infertility among these patients, it seems necessary to evaluate and present a cost-effective and available method with few complications, easy application, and further follow-up to eliminate infertility and increase pregnancy rate.

N-acetyl cysteine (NAC) (the acetylated variant of the amino acid L-cysteine) has been used as mucolytics in various respiratory, non-respiratory, and other diseases. This medication has antioxidant properties and reduces insulin resistance (3-5). Since insulin-resistance and hyperinsulinemia are the common causes of hyperandrogenism and anovulation, any agent that can reduce the insulin resistance can be beneficial in the treatment of infertility caused by PCOS (6).

The standard treatment of infertility due to PCOS is the administration of clomiphene citrate (1,2). This medication is a cost-effective treatment, which has an easy administration and relatively low side effects. Moreover, in this method, there is little need for ovarian assessment to obtain a response (2). However, 15 to 40% of the females with PCOS are resistant to clomiphene citrate (1,2). In addition, evidence has revealed the positive impacts of NAC along with clomiphene citrate on the treatment of

Received 16 May 2018, **Accepted** 9 August 2018, **Available online** 20 August 2018

[1]Department of Obstetrics and Gynecology, Neonatal and Maternal Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
***Corresponding Author:** Nayereh Ghomian, MD; Email: ghomiann@mums.ac.ir

infertility caused by PCOS (3,4). However, further studies are needed to evaluate the effects of this inexpensive medication more comprehensively, which is associated with few complications. With this background in mind, our study was designed to compare the pregnancy rate in females with PCOS treated with clomiphene alone and along with NAC.

Materials and Methods

This blinded randomized controlled clinical trial was conducted on the infertile patients with PCOS, who were candidates of ovulation induction by referring to Milad Infertility Clinic in Mashhad, Iran, in 2015. The patients were enrolled through the simple random sampling technique. According to the previous report by Abu Hashim et al (3) and using the formula for comparing 2 meanings with an alpha of 5% and a beta of 20%, the sample size was estimated as 33 individuals while considering a 10% rate for dropout.

The inclusion criteria consisted of (1) age of >18 years, (2) openness of at least one of the uterine tubes based on hysterosalpingogram imaging, (3) lack of endocrine diseases (e.g. hyperprolactinemia, hypothyroidism, and Cushing's syndrome), (4) normal semen analysis (spermogram) of the partner, and (5) confirmation of infertility due to PCOS based on the Rotterdam criteria by having at least two out of the three criteria, including oligomenorrhea or amenorrhea, clinical or laboratory hyperandrogenism, and detection of polycystic ovary in ultrasound. The exclusion criteria were a change in the diagnosis of the disease during the research period and participants' unwillingness to cooperate with us in conducting the study.

After spontaneous or progesterone-induced menstruation, on the third day of the menstrual cycle, the patients underwent transvaginal sonography with a 7.5 MHz probe. The ultrasound was performed to assess the ovary and endometrial thickness and rule out other pathologies. Afterwards, using a random number table, the patients were divided into two groups of control and intervention who received clomiphene alone and clomiphene along with NAC, respectively.

In the control group, 100 mg of clomiphene (i.e. two 50 mg pills, made by Iran Hormone Co., Iran) was administered from the third to seventh day of the menstrual cycle. On the other hand, the intervention group received 100 mg of clomiphene (i.e. two 50 mg pills) along with 1200 mg of NAC (i.e. two 600 mg pills, made by Fluid UI MUCIL Co., Iran) from the third to seventh day of menstrual cycle.

On the 12th day of the menstrual cycle, the patients underwent transvaginal sonography with a 7.5 MHz probe to be assessed in terms of the number and size of ovarian follicles and endometrial thickness. After the detection of at least an 18-mm follicle in the ultrasound, 5000 units of human chorionic gonadotropin (hCG, Pregnyl) was intramuscularly injected. The patients were required to have scheduled coitus 24 to 36 hours after the hCG injection. If the patients did not have their menses after 14 days, the beta-hCG test was performed according to which a positive result was regarded as pregnancy.

The ultrasound findings were assessed on the 12th day in the two groups in terms of the endometrial thickness as well as the maximum size and number of the right and left ovarian follicles. Furthermore, the frequency of positive beta-hCG test was evaluated 14 days after hCG injection.

Data analysis was carried out by normalizing the data using the one-sample Kolmogorov-Smirnov test (Lilliefors test), showing normally distributed data. The independent t test was utilized to compare the mean of quantitative data between the 2 groups. In addition, the numerical variables were analyzed using Pearson's chi-square test or Fisher exact test as appropriate. Data analysis was performed in the SPSS version 19.0. A P value of <0.05 was considered significant.

All the patients were informed about the objectives of the study prior to the study, and their informed consents were obtained. In addition, we ensured the confidentiality of their personal information. In this regard, the patients' data were entered into statistical analysis programs with codes and presented as a general result.

Results

The mean age of the participants was 28.6 ± 6.5 years. The demographic characteristics of the participants, including age, body mass index, duration of infertility, and history of infertility treatment, are presented in Table 1. According to this table, there was no statistically significant difference between the study groups. The ultrasound findings, including endometrial thickness on the third and 12th days, a number of ovarian follicles, and mean size of follicles, in the two study groups are shown in Table 2, revealing no significant difference between the two groups in this regard.

Out of the 66 participants, 42 (63.3%) patients had the indication of the injection of 5000 units of hCG, in a way that at least one follicle of ≥18 mm was observed in the ovaries of the patients. In addition, beta-hCG was positive for 12 (18.1%) subjects after follow-ups, 7 (21.2%) and 5 (15.1%) of whom were in the clomiphene/NAC and clomiphene groups, respectively. According to the results, the type of intervention showed no statistically significant difference in the frequency of indication of hCG injection and beta-hCG result ($P = 0.12$ and $P = 0.26$, respectively).

Discussion

This study aimed to compare the use of clomiphene alone and along with NAC in the pregnancy rate of the females with PCOS-related infertility. As the findings of this study revealed, the addition of NAC to the medical regimen of the patients had no significant impacts on their rate of pregnancy. This result would raise the controversy among

Table 1. Comparison of Underlying Variables Between the Two Groups of Clomiphene Plus N-acetyl Cysteine and Clomiphene Alone

	Clomiphene Plus N-acetyl cysteine (n=33)	Clomiphene Alone (n=33)	P Value
Age (y), mean±SD	28.7±6.9	28.5±6.2	0.93
Marriage duration (y), mean±SD	4.8±4.1	5.1±3.2	0.78
Body mass index (kg/m^2), mean±SD	24.5±3.0	25.33.5±	0.39
History of infertility treatment, No. (%)	9(42.4%)	14(27.3%)	0.15

Table 2. Comparison of Endometrial Thickness on the Third and Twelfth Days of the Menstrual Cycle, and Size and Number of Follicles Between the Intervention and Control Groups

	Clomiphene Plus N-acetyl cysteine (n=33)	Clomiphene alone (n = 33)	*P* Value
Endometrial thickness on the 3rd day (mm)	4.58±1.08	4.83±1.00	0.944
Endometrial thickness on the 12th day (mm)	7.47±1.60	7.58±2.10	0.810
Size of follicles (mm)	15.9±5.1	13.6±4.2	0.3
Numbers of follicles	1.8±0.9	1.56±0.9	0.07

the studies conducted in this area. The current study is the ninth clinical trial investigating the effectiveness of NAC in infertility caused by PCOS. However, our study is listed under the category of the studies that revealed the ineffectiveness of NAC.

In our research, the mean age of the participants was 28.5 years, which is in congruence with other studies. In this regard, the minimum and maximum mean ages reported in other studies were around 26 and 29 years, respectively (4-6). Likewise, the mean age of the subjects in several studies was within the mentioned age range (2,7-10). Endometrial thickness was another underlying variable investigated in this study. However, only 2 other studies have accurately and quantitatively estimated this variable. In the present study, the mean thickness of the endometrium in the evaluated participants was 4.7 mm, indicating no significant difference between the groups.

In a previous report by Rizk et al (10), the mean values of endometrial thickness were reported to be 4.9 and 5.9 mm in the control and intervention groups, respectively. Consistent with the present study, they did not find a significant difference between the study groups in terms of endometrial thickness. However, Salehpour et al (7) reported a totally different finding in this regard. In the mentioned study, the thickness of the endometrium was significantly greater in the intervention group at the beginning of the study, compared to the control group (6.6 mm versus 5.4 mm). Accordingly, at the end of the study, the intervention group had a higher pregnancy rate.

Studies have shown that NAC increases cellular antioxidants while significantly decreasing glutathione (11). Therefore, NAC is able to improve the activity of the insulin receptor in the human blood cells and secretion of insulin in response to glucose (12).

The improved function of insulin receptors in the patients with hyperinsulinemia can lead to a secondary reduction in the second-phase glucose response of β-cell to glucose tolerance test. Decreased insulin level of blood flow also results in an apparent decrease in testosterone and free androgen levels in the females who respond to the treatment (2,12).

Nevertheless, three randomized clinical trials, conducted on a total of 292 patients, have presented strong evidence demonstrating the ineffectiveness of NAC in female patients with clomiphene citrate-resistant PCOS (1-3). However, the important difference between these studies and our research, despite the consistency in results, was the study population.

In the three mentioned studies, the effectiveness of NAC in patients with clomiphene citrate-resistant PCOS was compared with a placebo. Nonetheless, in the present study, we compared the effectiveness of clomiphene/NAC with clomiphene alone in the females with infertility caused by PCOS. In a prospective controlled trial carried out by Youssef et al (1) in 2006 on the individuals with clomiphene citrate-resistant PCOS, no statistical difference was reported in the rate of clinical pregnancy between the study groups.

However, in the previously mentioned study, the researchers ascribed the ineffectiveness of NAC in infertility to their small sample size. They stated that the clinical application of NAC had positive effects on the improvement of infertility in the patients. Similarly, in the present study, we utilized a small sample size, which could be one of the causes of the observation of the insignificant difference in the mean thickness of endometrium or diameter of ovarian follicles.

In another study, Elnashar et al (2) investigated the effect of NAC and metformin on ovulation rate in the women with PCOS resistant to clomiphene citrate. Similar to the research by Yousef, the mentioned study had an apparent difference with our study in terms of the study population. However, the mentioned study demonstrated that NAC did not lead to a better ovulation rate, which is

in line with ours.

Furthermore, they reported the ovulation rates to be 51.6% and 6.7% in the metformin and NAC groups, respectively, which was indicative of a significant difference between the study groups. In our study, despite the fact that a greater number of individuals treated with NAC showed a positive beta-hCG result, compared to the controls (21.2% versus 15.1%), this difference was not significant.

However, the lack of a statistically significant difference between the groups cannot be simply interpreted as the weak impact of NAC intervention on pregnancy rate. In this regard, the duration of follow-up is regarded as an important factor, which has been identified as one of the most serious limitations of the previous studies conducted in this area. It is assumed that the increased duration of follow-ups might lead to different results. This issue was pointed out in a review study published in 2015 (11). In the mentioned study, it was marked that all of the studies conducted on this subject had short-term follow-ups (i.e. a maximum of three months) with no long-term data focusing on the comparative effects of NAC on important treatment outcomes, such as the initiation of regular menstruation (11). In line with our study, in a research conducted by Abu Hashim et al (3), it was concluded that the combined administration of clomiphene and metformin had more positive impacts on inducing ovulation and pregnancy in the women with clomiphene citrate-resistant PCOS, compared to the combined use of NAC and clomiphene citrate. However, the mentioned study employed a larger sample size.

Therefore, including the present article, there are four clinical trials (1-3), revealing the ineffectiveness of NAC, at least in the improvement of infertility. On the other hand, there are five studies indicating the effectiveness of NAC in the improvement of infertility. In this regard, Maged et al (4) demonstrated that NAC, as a complementary medication consumed with clomiphene citrate, had positive effects on the endometrial thickness, inducing ovulation, number of follicle, and pregnancy in PCOS patients. In addition, Salehpour et al (7) reported that the clomiphene citrate/NAC group had significantly more follicles larger than 18 mm and higher mean endometrial thickness on the hCG administration day, compared to the clomiphene citrate group. Moreover, the ovulation and pregnancy rates were significantly higher in the clomiphene citrate/NAC group, compared to the control group.

In another study, Nasr (8) investigated the effectiveness of NAC administration as a complementary treatment after unilateral laparoscopic ovarian drilling (LOD) in the females who had PCOS resistant to clomiphene citrate. They concluded NAC as a new complementary treatment method after performing LOD, which could be used in the improvement of pregnancy outcomes. In addition, in the studies conducted by Badawy et al (9) and Rizk et al (10), NAC was reported to have positive impacts on inducing or improving ovulation in the patients with PCOS. In a systematic review and meta-analysis (11), none of these studies had all of the high-quality criteria observed in clinical trials, including randomization, blinding, control group and expression of possible bias. In addition, none of the studies adjusted the difference in the index base.

Furthermore, in four studies, the sperm quality test of the spouse, which is a vital indicator of pregnancy rate, was not reported (2,13,14). However, this criterion was fully reported in the present study. Additionally, just three studies analyzed the normality of the sperms (2,13,15). Moreover, in the majority of the conducted studies, the effect of NAC was evaluated in a short follow-up (8). It should be mentioned that the difference in the protocol of NAC administration was various in studies, which could be the cause of differences in the final results.

Despite a large number of studies investigating this issue, according to the results of a recently published review article, there is still a need to perform more clinical trials on a large number of women with PCOS resistant or non-resistant to clomiphene citrate to obtain a clear perspective about this issue. Therefore, it is recommended that meta-analysis studies be performed with the aim of finding a suitable dose for obtaining the highest effect of NAC on the treatment of infertility in patients with PCOS.

Conclusions

According to the findings of the present study, it seems that adding NAC to clomiphene treatment had no positive impacts on the pregnancy rate of the patients with PCOS-related infertility. Given the discrepancy in the findings of the studies conducted in this area, it seems that further studies are required in this regard.

Conflict of Interests

Authors declare that they have no conflict of interests.

Ethical Issues

Ethics Committee of Mashhad University of Medical Sciences, Mashhad, Iran approved this study (code: Ir.mums.fm.rec.1394.347). It has also been registered in Iranian Registry of Clinical Trials (identifier: IRCT2016042727638N1).

Financial Support

Deputy of research of Mashhad University of Medical Sciences supported the study.

Acknowledgments

We greatly appreciate the support of deputy of research of Mashhad University of Medical Sciences.

References

1. Youssef G, Makin B, Ali AM, Waly M, Alaa N, Abou-Setta A. N-acetyl-cysteine in anovulatory women: The impact of

postcoital test. Middle East Fertil Soc J. 2006;11(2):109-112.
2. Elnashar A, Fahmy M, Mansour A, Ibrahim K. N-acetyl cysteine vs. metformin in treatment of clomiphene citrate-resistant polycystic ovary syndrome: a prospective randomized controlled study. Fertil Steril. 2007;88(2):406-409. doi:10.1016/j.fertnstert.2006.11.173
3. Abu Hashim H, Anwar K, El-Fatah RA. N-acetyl cysteine plus clomiphene citrate versus metformin and clomiphene citrate in treatment of clomiphene-resistant polycystic ovary syndrome: a randomized controlled trial. J Womens Health (Larchmt). 2010;19(11):2043-2048. doi:10.1089/jwh.2009.1920
4. Maged AM, Elsawah H, Abdelhafez A, Bakry A, Mostafa WA. The adjuvant effect of metformin and N-acetylcysteine to clomiphene citrate in induction of ovulation in patients with Polycystic Ovary Syndrome. Gynecol Endocrinol. 2015;31(8):635-638. doi:10.3109/09513590.2015.1037269
5. Cheraghi E, Soleimani Mehranjani M, Shariatzadeh MA, Nasr Esfahani MH, Ebrahimi Z. Co-Administration of Metformin and N-Acetyl Cysteine Fails to Improve Clinical Manifestations in PCOS Individual Undergoing ICSI. Int J Fertil Steril. 2014;8(2):119-128.
6. Sacchinelli A, Venturella R, Lico D, et al. The Efficacy of Inositol and N-Acetyl Cysteine Administration (Ovaric HP) in Improving the Ovarian Function in Infertile Women with PCOS with or without Insulin Resistance. Obstet Gynecol Int. 2014;2014:141020. doi:10.1155/2014/141020
7. Salehpour S, Sene AA, Saharkhiz N, Sohrabi MR, Moghimian F. N-Acetylcysteine as an adjuvant to clomiphene citrate for successful induction of ovulation in infertile patients with polycystic ovary syndrome. J Obstet Gynaecol Res. 2012;38(9):1182-1186. doi:10.1111/j.1447-0756.2012.01844.x
8. Nasr A. Effect of N-acetyl-cysteine after ovarian drilling in clomiphene citrate-resistant PCOS women: a pilot study. Reprod Biomed Online. 2010;20(3):403-409. doi:10.1016/j.rbmo.2009.12.012
9. Badawy A, State O, Abdelgawad S. N-Acetyl cysteine and clomiphene citrate for induction of ovulation in polycystic ovary syndrome: a cross-over trial. Acta Obstet Gynecol Scand. 2007;86(2):218-222. doi:10.1080/00016340601090337
10. Rizk AY, Bedaiwy MA, Al-Inany HG. N-acetyl-cysteine is a novel adjuvant to clomiphene citrate in clomiphene citrate-resistant patients with polycystic ovary syndrome. Fertil Steril. 2005;83(2):367-370. doi:10.1016/j.fertnstert.2004.07.960
11. Thakker D, Raval A, Patel I, Walia R. N-acetylcysteine for polycystic ovary syndrome: a systematic review and meta-analysis of randomized controlled clinical trials. Obstet Gynecol Int. 2015;2015:817849. doi:10.1155/2015/817849
12. Fulghesu AM, Ciampelli M, Muzj G, et al. N-acetyl-cysteine treatment improves insulin sensitivity in women with polycystic ovary syndrome. Fertil Steril. 2002;77(6):1128-1135. doi:10.1016/S0015-0282(02)03133-3
13. Salehpour S, Tohidi M, Akhound MR, Amirzargar N. N acetyl cysteine, a novel remedy for poly cystic ovarian syndrome. Int J Fertil Steril. 2009;3(2):66-73.
14. Gayatri K, Kumar JS, Kumar BB. Metformin and N-acetyl Cysteine in Polycystic Ovarian Syndrome--A Comparative Study. Indian J Clin Med. 2010;1:7-13. doi:10.1177/117739361000100002
15. Oner G, Muderris, II. Clinical, endocrine and metabolic effects of metformin vs N-acetyl-cysteine in women with polycystic ovary syndrome. Eur J Obstet Gynecol Reprod Biol. 2011;159(1):127-131. doi:10.1016/j.ejogrb.2011.07.005