ABSTRACT

Aim: The objective of this work was to assess the impact of adding oral N-acetyl cysteine (NAC) to clomiphene citrate (CC) on ovulation induction outcomes in women diagnosed with polycystic ovary syndrome (PCOS).

Material and Methods: In this controlled clinical trial was carried out between June 2013 and August 2015. Patients were carefully chosen from the outpatient clinic of obstetrics and gynecology at Benha University Hospital in addition to private centers. 113 infertile women with PCOS were evaluated for eligibility; 13 patients were excluded (they did not give approval consent). 100 PCOS infertile females were randomly recruited then divided into two equal groups for induction of ovulation. 50 women in group I (CC+NAC) received CC 50 mg/b.i.d. combined with NAC 1200 mg/d while patients in group II (CC only) received CC alone for 5 days starting day 3 of their menstrual cycle. On the twelfth day of the menstrual cycle within the appearance of at least one follicle measured (18–20-mm) by ultrasound examination, 10 000 U hCG was given intramuscularly, and scheduled intercourse was encouraged 36 h after hCG therapy. Outcome measures included the number of mature follicles, serum E2 and serum progesterone levels, in addition to the endometrial thickness. The development of clinical pregnancy was also analyzed and compared between the groups.

Results: The number of follicles (>18 mm) on the day of hCG therapy and ovulation rates showed significant rise in the CC+NAC group (P-value < 0.001). The mean endometrial thickness and pregnancy rate were also significantly increased in the CC+NAC group (P-value=0.024 and 0.002, respectively). No adverse events were reported and no cases of ovarian hyperstimulation syndrome were seen among the patients receiving NAC.

Conclusion: NAC as an adjuvant to CC for induction of ovulation that may enhance ovulation and pregnancy rates in PCOS with some helpful effect on endometrial thickness. NAC is safe, well-tolerated, and relatively inexpensive primary adjuvant treatment to boost the ovulation and pregnancy outcomes in PCOS patients.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a familiar disorder clearly identified throughout the endocrine health prob-
lems, affecting around 5% to 15% of women during their reproductive period (Shannon & Wang, 2012, Yildiz et al., 2012). PCOS is chiefly correlated with anovulation and subsequent infertility, insulin resistance, and hyper-androgenism that prompt some metabolic disorders such as diabetes and cardiovascular diseases (Huang et al., 2010, Pangaribuan et al., 2012). PCOS comprises a wide range of symptoms starting at the early prepubertal time and advancing till the menopause. The phenotypic expression is variable through time and continuously influenced by various internal factors including ovarian-adrenal steroidogenesis and insulin resistance in addition to external factors such as quality and quantity of food and life style. To a considerable extent, etiology of PCOS has remained confusing although it has been cleared that elevated androgen, and insulin-resistance (IR) lies at the core of its pathophysiology (Livadas & Diamanti-Kandarakis, 2013). Clomiphene citrate (CC), the conventional first-line medication for ovulation induction in anovulatory women, has variable estimated success rates. Its potency is noticed to be the lowest in women diagnosed with PCOS and insulin resistance. There is substantial evidence validating that insulin sensitizers reduce hyperandrogenism, and hyper-insulinemia in patients with PCOS, and are notably adequate for ovulation induction with those patients (Kolodziejczyk et al., 2000, Kashyap et al., 2004). N-acetylcysteine (NAC) is traditionally used as a safe mucolytic agent, has been emerged to have an impact on both the insulin secretion in pancreatic b-cells and additionally the regulation of insulin receptors in human erythrocytes (Santini et al., 1990). NAC also has an antioxidant effects by raising the intracellular levels of reduced glutathione, (Wentzel & Eriksson, 2002) along with its protective action on the vascular endothelial damage in noninsulin-dependent diabetics has been confirmed (Pieper & Siebeneich, 1998). As various number of reports has documented the roven efficacy of NAC administration in enhancing insulin potency and better improvement in ovulation results in patients with PCOS (Saha et al., 2013).

This study was designed to assess the impact of oral NAC administration in combination with CC on induction of ovulation outcomes in PCOS patients.

**PATIENTS AND METHODS**

A Prospective controlled double-blind, randomized clinical trial was conducted between June 2013 and August 2015. Patients were carefully chosen from the outpatient clinic of obstetrics and gynecology at Benha University Hospital in addition to private centers. 113 women were included; 13 patient were excluded (they did not give approval consent) so 100 PCOS infertile patients enrolled in this study. At the time of diagnosis, the patients fulfilled the inclusion criteria would randomly be stratified to two groups:

I. **Group I (NAC+CC):** included (50 cases) received clomiphene citrate (CC) 50 mg/bid. plus oral N-acetyl cysteine (NAC) 1200 mg/d for induction of ovulation for 5 days starting on day 3 of the cycle.

II. **Group II (CC only):** included (50 cases) received clomiphene citrate (CC) 50 mg/bid. alone for induction of ovulation for 5 days starting on day 3 of the cycle.
Randomization protocol: Women selected were randomized between oral N-acetyl cysteine (NAC+CC) group and clomiphene citrate (CC only) group. Randomization was performed using a computer-generated randomization list. Sealed envelopes with treatment instructions were opened on the first day of ovulation induction.

Inclusion criteria: females aged between 20 to 35 Years, Infertility period less than 10 years, Body mass index (BMI) < 35 kg/m², patient tubal patency confirmed by hysterosalpingography or laparoscopy. Partner’s normal semen analysis results (total volume > 2cc, concentration > 20 million/ml, total motility > 50%, normal morphology > 14%).

Exclusion criteria: Thyroid dysfunction, hyperprolactinemia, hypercorticism, history of ovarian cyst formation (>6 cm), history of visual disturbance caused by CC, history of asthma and or allergy to medications, Patients who had received medications affecting metabolism of glucose for at least 3 months prior the study, also, Patients who had received any hormonal medicines (apart from progesterone for withdrawal bleeding) and patients or their male partners had any sexual dysfunction interfering with successful intercourse.

Diagnostic criteria: Diagnosis of PCOS was based on the criteria of the ESHRE/ASRM Rotterdam consensus meeting in 2003 (The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group, 2004), which broadened the previous NIH classification of 1990 (Moran et al., 2003; Madnani et al., 2013).

Outcome measures: The number of follicles >18 mm, the serum E2 concentration, serum Progesterone and the endometrial thickness on the day of HCG injection in addition to the ovulation and clinical pregnancy rates.

Interventions: 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 (TSH), E2 and prolactin levels assessment were performed for all patients who were candidate for induction of ovulation. At that point, patients were stratified into two groups. In the first group (NAC group), from day 3 until day 7 of the menstrual cycle; patients got 100 mg CC in addition to 1200 mg N-acetylcysteine. NAC was given to the subjects as powder embedded into little pockets to be diluted into one glass of water and taken orally two divided daily doses. In the second group (CC only ) 50 mg twice daily doses CC. 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. within sight of no less than one follicle with an (18–20-mm ) width in ultrasound assessment, 10 000 U hCG was implanted intramuscularly, and coordinated intercourse was prompted 36 h after hCG infusion. At the same day, serum progesterone and E2 are measured while Serum b-hCG level was estimated on the day sixteenth. With two subsequent positive b-hCG levels (no less than 2 days separated) another transvaginal ultrasound inspection was done on the 6th week of gestation to confirm the clinical pregnancy.
Statistical analysis: Data were analyzed by Statistical Program for Social Science (SPSS) Ver. 20 Quantitative. Data were expressed as the mean± standard deviation (SD).

Ethical consideration: Consent for our study was obtained from the hospital's ethical committee; and informed consent was gotten from patients after adequate providing of information about the study necessities, purpose, and safety profile.

RESULTS

A total of 113 were included; 13 patient were excluded (they did not give approval consent) so 100 patients were randomly divided into two equal groups; group one CC+NAC [n=50], group two CC only [n=50]. Comparing the demographic characteristics in both groups revealed that the two groups were matching regarding age, BMI and duration of infertility (Table 1). Years of infertility were estimated (4.31± 1.72) in the NAC group compared to (4.28 ± 1.64) in the CC only group with no significant difference between the groups.

No significant difference was reported between the two groups in relation to, basal LH/FSH ratio, ovarian volume, serum prolactin, TSH. The mean±SD serum E2 level was (55.31± 30.46) in the first group in comparison to (54.74±28.21) with no reported significant difference between them as shown in (Table 2).

The number of follicles measuring >18 mm on the day of hCG administration was 1.91 ± 0.75 mm and 0.62 ± 0.31 mm in the first and second groups respectively which showed significant difference when comparing the two groups. Serum E2 and progesterone at the time of HCG administration showed significant rise in the NAC group upon comparison to CC only group. Ovulation rates and mean endometrial thickness were also significantly increased among the CC+NAC group (P-value=0.0001). 62 % ovulation rate was seen in the NAC group while 29 % in the CC only group which showed statistically significant difference. The mean pregnancy rate were also significantly higher in the CC+NAC group (P-value=0.024 and 0.002, respectively). Clinical pregnancy rate was 36 % in the NAC group compared to only 8 % in the CC only group with statistical significant difference in favor of NAC group. No adverse side-effects and no cases of ovarian hyper-stimulation syndrome were observed in the group receiving NAC (Table 3).

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

<table>
<thead>
<tr>
<th>Demographic characteristics</th>
<th>CC+NAC (n=50)</th>
<th>CC only (n=50)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>27.13±3.56</td>
<td>26.84±3.67</td>
<td>NS</td>
</tr>
<tr>
<td>BMI</td>
<td>26.58±1.95</td>
<td>26.77±2.23</td>
<td>NS</td>
</tr>
<tr>
<td>Infertility duration (years)</td>
<td>4.31±1.72</td>
<td>4.28±1.64</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD. NS= not significant.
In this controlled clinical trial study, we tried to assess the upshot of oral N-acetyl cysteine administration as an adjuvant to clomiphene citrate on induction of ovulation outcomes in patients with polycystic ovary syndrome (PCOS). Considering our results, it revealed a significantly better ovulation rate in PCOS patients who got NAC as an adjuvant to CC for induction of ovulation. Since the insulin resistance has been appeared to be a reason for CC dissatisfaction in both fat and non-obese PCOS patients (Moghetti et al., 2000). The potential insulin-sensitizing effects of NAC may lead to better induction of ovulation in these patients. 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 may influence insulin receptor action and resulted in an increase of cell glucose utilization which is a pointer to the insu-
lin affectability state (Soltan-Sharifi et al., 2007). 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 (Fulghesu et al., 2002). A significant fall in testosterone level and free androgen index was also demonstrated with NAC treatment in PCOS patients in their study. 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 (Kilic-Okman and Kucuk, 2004). It has additionally been demonstrated that prolonged treatment with NAC in addition to L-arginine may reestablish gonadal capacity in PCOS in a relationship with a modification in insulin sensitivity (Masha et al., 2009). 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. In addition to its insulin-sensitizing and androgen reducing effects some other biological effects of NAC such as anti-apoptotic and antioxidant effects (Odetti et al., 2003). Inhibition of phospholipid metabolism, pro inflammatory cytokine release, and protease activity, may prompt better folliculogenesis and ovulation rate in PCOS patients. As far as anyone is concerned, just a predetermined number of studies have assessed the induction of ovulation results in PCOS patients treated with NAC. Elnashar et al. demonstrated that NAC isn't a compelling solution to stimulating ovulation in CC-resistant PCOS patients (Elnashar et al., 2007), 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 (Badawy et al., 2007). A recent study has found a significant increase in ovulation, pregnancy rates and better reproductive outcome in PCOS patients who received NAC after unilateral laparoscopic ovarian drilling (Nasr, 2010). It has been showed in the previous study by Abu Hashim et al. that combination of CC and NAC for induction of ovulation significantly increases the E2 level at the time of triggering with HCG, ovulation and pregnancy rate in women with CC-resistant PCOS compared to the CC plus placebo group (Abu Hashim et al., 2010). Our data support the results of their study, but based on our findings, NAC might be beneficial as an adjuvant to CC for induction of ovulation in a more extended scope of PCOS patients and not merely constrained to CC-resistant PCOS ladies. No antagonistic impacts of NAC were seen among PCOS patients and not merely constrained to CC-resistant PCOS ladies. No antagonistic impacts of NAC were seen among PCOS patients in both studies, and the medication appeared to be safe and well-tolerated by all subjects. It has been stated that CC may have an adverse 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 (Sh Tehrani Nejad et al., 2008). These adverse effects of CC on the endometrium may explain in part the relatively poor pregnancy rates associated with CC despite the high incidence of ovulation. In contradiction of the study by Rizk et al., which did not reveal any significant change in endometrial thickness (Rizk et al., 2005), 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 (Sekhon et al., 2003) 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.

CONCLUSION

NAC as an adjuvant to CC for ovulation of ovulation can enhance the ovulation and pregnancy rates in PCOS patients and may likewise 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. It could be used an alternative to other insulin-sensitizing agents like metformin or troglitazone.

REFERENCES


التحريض على التبويض باستخدام أسيتيل سيستينين
في حالات متلازمة كيسات المبيض

يهاب بركات
قسم التوليد وأمراض النساء-كلية الطب - جامعة بنها

الهدف: كان الهدف من هذا العمل هو تقديم الدعم إضافة عقار أسيتيل سيستينين إلى مراقبة التحريض التبويض لدى النساء اللاتي تم تشخيصهن بمتلازمة كيسات المبيض.

المصادر والطرق البحث: هذه التجربة السريرية الخاضعة للرقابة تم إجراؤها بين Nhưng 2013-أغسطس 2015. تم اختيار المرضى بعناية من المريضين الذين يعانون من متلازمة كيسات المبيض. تم تشكيل 113 امرأة مصابة بالمرض ومتلازمة كيسات المبيض تم استبعاد 13 مريضة (لم يعطوا الموافقة على إجراء البحث). تم اختيار 100 من المريضات الذين بحثت بشكل عشوائي ثم تم تقييمهم في مجموعتين متساويتين للتحريض على التبويض. تم إعطاء 50 مريضة في المجموعة الأولى أسيتيل سيستينين بالإضافة إلى عقار أسيتيل سيستينين عن طريق الفم في حين تلقى المريضات في المجموعة الثانية أسيتيل سيستينين فقط لمدة 5 أيام ابتداء من اليوم الثالث من الدورة الشهرية. في اليوم الثاني عشر من الدورة الشهرية أخذت الموجات فوق الصوتيات وتبويض وجود برو Soda واحدة ناضجة على الأقل تم إعطاء 1000 وحدة من عقار الهرمون المشيمي البشري في العضل، وتشجيع الجماع المتكرر خلال 36 ساعة بعد العلاج. وشملت النتائج على عدد البوسانتات الناضجة، مستوى هرمون الاستروجين و البروجسترون في الدم بالإضافة إلى سمند بطاقة الرحم. كما تم تسجيل حالات حدوث الحمل والمقارنة بين المجموعات.

النتائج: أظهر عدد البوسانتات الناضجة ومعدلات التبويض ارتفاعًا ملحوظًا في المجموعة الأولى. كما زاد متوسط سمند بطاقة الرحم ومعدل الحمل بشكل ملحوظ في

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The study on the first group also showed no evidence of any complications, and no cases of multiple pregnancy were recorded.

The study concludes: Ectopic pregnancy may be a complication of gonadotropin stimulation therapy. The study shows that the incidence of ectopic pregnancy is significantly higher in cases of multiple pregnancy. An increase in pregnancy rates following the use of assisted reproductive techniques, especially in cases of multiple pregnancy. In cases of multiple pregnancy, the study concludes that the use of assisted reproductive techniques is essential for the treatment of patients with multiple pregnancy.