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EFFECT OF N-ACETYLCYSTEINE AS AN ADJUVANT TO CLOMIPHENE CITRATE FOR INDUCTION OF OVULATION IN PATIENTS WITH POLYCYSTIC OVARY SYNDROME

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ABSTRACT

Objective: *The aim of this study was to evaluate the effect of oral N-acetyl cysteine (NAC) administration as an adjuvant to clomiphene citrate (CC) on induction of ovulation outcomes in patients with polycystic ovary syndrome (PCOS).* ***Patient and Methods:*** *In this placebo-controlled double-blind randomized clinical trial, 200 PCOS infertile patients were randomly divided into two groups for induction of ovulation. Patients in group 1 received CC 100 mg/d plus NAC 1.2 g/d and patients in group 2 received CC plus placebo for 5 days starting at day 3 of the cycle. On the 12th day of the menstrual cycle in the presence of at least one follicle with an 18–20-mm diameter in ultrasound evaluation, 10 000 U hCG was injected intramuscularly and timed intercourse was advised 36 h after hCG injection. Serum b-hCG level was measured on the 16th day after hCG injection.* ***Results:*** *The number of follicles >18 mm on the day of hCG administration and ovulation rates were significantly higher among the CC+NAC group(P-value<0.0001). The mean endometrial thickness and pregnancy rate were also significantly higher in the CC+NAC group (P-value=0.022 and 0.002, respectively). No adverse side-effects and no cases of ovarian hyperstimulation syndrome were observed in the group receiving NAC.* ***Conclusion:*** *NAC as an adjuvant to CC for induction of ovulation can improve the ovulation and pregnancy rates in PCOS patients and may also 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.*

KEYWORDS: *clomiphene citrate, N-acetylcysteine, ovulation, polycystic ovary syndrome, pregnancy rate.*

INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders of women at reproductive age and the major cause of anovulatory infertility.[1] It is one of the most common endocrine disorders, affecting approximately 5% to 15% of women of reproductive age.[2] PCOS is mainly associated with anovulation, infertility, insulin resistance, and Hyperandrogenism leading to metabolic disorders such as diabetes and cardiovascular diseases.[3]

Treatment remains a challenge for women with PCOS. Although clomiphene citrate (CC) is the first-line of treatment for chronic anovulation among women with PCOS, failure to ovulate after receiving 150mg/ day is common and occurs in approximately 15% to 40% of women.[4] For those who do not respond to CC, there are very few therapies that can be tried before moving on to gonadotropin therapy or laparoscopic ovarian drilling (LOD).

CC treatment has shown discrepancy between ovulation rates (75% to 80%) and conception rates (30% to 40%) unlike LOD treatment used in women with CC resistant PCOS.[5] The discrepancy might persist to a certain extent with gonadotropin treatment as well.[6] Insulin-sensitizing agents have been explored for treating the underlying cause of disorders associated with insulin resistance.

Metformin, a widely used oral biguanide for treating type 2 diabetes, decreases the levels of insulin and androgens and increases the level of sex-hormone-binding globulin, thereby improving the endocrine parameters such as glucose tolerance and ovulation rates in women with PCOS.[7] However, a recent Cochrane review revealed that even though metformin was associated with improved clinical pregnancy and ovulation rate, it did not improve live birth rates when used alone or in combination with clomiphene or when compared with clomiphene.[8]

Therefore, there is need for developing therapeutic options for treating the women with PCOS. N-Acetyl cysteine (NAC) is a commonly used safe mucolytic drug; In addition, NAC increases the cellular levels of antioxidant and reduces glutathione at higher doses. Therefore, NAC has a potential to improve insulin receptor activity in human erythrocytes and improve insulin secretion in response to glucose.[9]

Improvement in insulin receptor activity in hyperinsulinemic subjects can lead to a secondary decrease in the β-cell responsiveness to the oral glucose tolerance test. Decreased levels of circulating insulin can lead to significant reduction in Testosterone levels and free androgen index in women responding to the treatment.[10]

Advantages resulting from administration of NAC include prevention of endothelial damage resulting from oxidants in non-insulin-dependent adult diabetic subjects and biological effects such as, protection against focal ischemia, inhibition of phospholipid metabolism inhibition, proinflammatory cytokine release, and protease activity.[10]

Therefore, it was suggested that the above effects exerted by NAC at the ovarian level may be as beneficial as its insulin-enhancing effects in inducing ovulation. In the absence of effective treatment options for PCOS, establishment of data on new options like NAC as monotherapy or supportive therapy may provide valuable information. There is no systematic review assessing effectiveness of NAC in PCOS.

In recent years a limited number of studies has shown the possible benefits of NAC administration in improving insulin sensitivity and better induction of ovulation outcomes in patients with PCOS.[9]

PATIENTS AND METHODS

200 patients were selected from the outpatient clinic of obstetrics and gynecology at El Hussein Hospital. This Prospective placebo-controlled double-blind randomized clinical trial study was conducted between June 2015 and December 2016. It involved 200 PCOS infertile patients enrolled in this study.

At time of diagnosis, the patients met the inclusion criteria were assigned into two groups: The 1st group: included 100 cases were received clomiphene citrate (CC) 100 mg/d plus oral N-acetyl cysteine (NAC) 1.2 g/d for induction of ovulation for 5 days starting at day 3 of the cycle. The 2nd group: included 100 cases were received clomiphene citrate (CC) 100 mg/d plus placebo for induction of ovulation for 5 days starting at day 3 of the cycle.

Women selected were randomized between oral N-acetyl cysteine (NAC) group and a placebo group. Randomization was done with the use of a computer-generated randomization list. Sealed envelopes with treatment allocation instructions were opened on the first day of ovulation induction by a nurse who assigned participants to their groups. Ethical consideration:

The details of study were explained to patients and informed consents were taken.

INCLUSION CRITERIA

Age: 20 Years to 35 Years. Infertility duration less than 10 years. Body mass index (BMI) < 35 kg/m2. Both patient tubes 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, hyperprolactin-aemia, hypercorticism, history of large ovarian cyst formation (>6 cm), history of visual disturbance caused by CC and finally history of asthma and or allergy to medications. Patients who had received any hormonal medications (except progesterone for withdrawal bleeding) or medications affecting glucose metabolism for at least 3 months before the study and patients or their male partners had any sexual dysfunction interfering with successful intercourse were excluded.

Outcome measures

The number of follicles >18 mm, the serum E2 concentration, serum P and the endometrial thickness on the day of HCG administration, 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 (all measured by immunoreactive multianalysis) were performed for all patients who were candidates for induction of ovulation.

Then patients were randomly divided into two groups. In the first group, patients received 100 mg CC plus 1200 mg NAC from day 3 until day 7 of the menstrual 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.

In the second group in addition to 100 mg daily CC, patients received a placebo (oral rehydration solution [ORS] powder) from day 3 until day 7. ORS powder was given to the subjects in the same pockets as NAC for two daily divided doses. On the 12th 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, 10 000U hCG was injected intramuscularly and timed intercourse was advised 36 h after hCG injection. At the same day, serum progesterone and E2 are measured. Serum b-hCG level was measured on the 16th day after hCG injection. With two serial positive b-hCG levels (at least 2 days apart) another transvaginal ultrasound examination was performed on the 6th week of gestation to determine the clinical pregnancy.

Statistical analysis

Data were analyzed using Statistical Program for Social Science (SPSS) version 18.0. Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

RESULTS

Table 1: Baseline variables in the case and control groups:

Variable	CC+NAC (n=100)	CC+Placebo (n=100)	P-value
Age (years)	27.97± 3.46	27.84± 3.77	NS
BMI	26.69± 1.95	26.77± 2.15	NS
Infertility duration (years)	4.25± 1.88	4.26 ± 1.72	NS
Basal LH/FSH ratio	1.93± 0.36	1.90 ± 0.83	NS
Basal Ovarian volume	9.92± 3.12	9.62 ± 4.34	NS
Basal Prolactin	15.77± 8.55	15.06± 8.16	NS
Basal TSH	2.15± 3.23	2.06± 1.66	NS
Basal E2	56.27± 31.56	54.62± 24.22	NS

Table 2: Induction of ovulation outcomes in the case and control groups

Variable	CC+NAC (n=100)	CC+Placebo (n=100)	P-value
the number of follicles >18 mm	1.84± 0.76	0.53 ± 0.33	< 0.0001
Serum E2 at the time of HCG	270.06 ± 288.25	120 ± 10	< 0.0001
Serum P at the time of HCG	5.10± 4.18	1.80 ± 2.20	< 0.0001
The endometrial thickness at the time of HCG	6.49± 4.18	5.47± 1.43	0.022
the ovulation rate	49%	16%	>0.0001
clinical pregnancy	18%	4%	0.002

A total of 200 patients were randomly divided into two groups (group one CC+NAC [n=100], group two CC+ Placebo [n=100]). There were no statistically significant baseline differences between the two groups in age, duration of infertility, BMI, basal LH/FSH ratio, ovarian volume, prolactin, TSH, E2 of patients (Table 1). As shown in Table 2, The number of follicles >18 mm on the day of hCG administration and ovulation rates were significantly higher among the CC+NAC group (P-value<0.0001).

The mean endometrial thickness and pregnancy rate were also significantly higher in the CC+NAC group (P-value=0.022 and 0.002, respectively). No adverse side-effects and no cases of ovarian hyperstimulation syndrome were observed in the group receiving NAC.

DISCUSSION

In this prospective placebo-controlled double-blind randomized clinical trial study, we tried to evaluate the effect of oral N-acetyl cysteine (NAC) administration as an adjuvant to clomiphene citrate (CC) on induction of ovulation outcomes in patients with polycystic ovary syndrome (PCOS).

There were 18 (18%) and 4 (4%) clinical pregnancies in the NAC and control groups, respectively (absolute difference 14%) which indicates extremely statistically significance between groups. Ovulation rates were 49% and 16% in the NAC and control groups, respectively (absolute difference 33%) which indicates extremely statistically significance between groups. The endometrial thickness on the day of HCG administration, the mean was (6.49±4.18 & 5.47±1.43) respectively, there was statistically significant difference between groups.

Serum P at the time of HCG administration the mean was (5.10±4.18 & 1.80±2.20) respectively, there was extremely statistically significance between groups. Serum E2 at the time of HCG administration, the mean was (270.06 ± 288.25 & 120.00 ± 10.00) respectively, there was extremely statistically significance between groups. The number of follicles >18 mm, the mean was

(1.84±0.76 & 0.53±0.33) respectively, there was extremely statistically significance between groups.

Based on our data we have found a significantly better ovulation rate in PCOS patients who received NAC as an adjuvant to CC for induction of ovulation. Since the insulin resistance has been shown to be a cause of CC failure in both obese and non-obese PCOS patients[11], the potential insulin-sensitizing effects of NAC may lead to better induction of ovulation in these patients.[12]

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 probably influences insulin receptor activity and results in an increase of cellular glucose consumption which is an indicator of the insulin sensitivity state.[12,13]

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[9]. A significant fall in testosterone level and free androgen index was also demonstrated with NAC treatment in PCOS patients in their study.[9] KilicOkman 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.[14]

In other study, it also was observed a significant decrease in weight, BMI, waist/hip ratio, fasting blood sugar, serum insulin, total cholesterol, low-density lipoprotein (LDL) levels, and homeostasis model assessment insulin resistance (HOMA-IR) index after a 6-week treatment with NAC in PCOS patients.[12] High-density lipoprotein (HDL) levels were also elevated significantly and NAC improved the lipid profile, hormonal levels, and ovulation status in women with PCOS in that study.[12]

It has also been shown that prolonged treatment with NAC plus L-arginine might restore gonadal function in PCOS in association with an improvement in insulin sensitivity.[15] 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[16], inhibition of phospholipid metabolism, proinflammatory cytokine release, and protease activity[17], may lead to better folliculogenesis and ovulation rate in PCOS patients. To our knowledge, only a limited number of studies have evaluated the induction of ovulation outcomes in PCOS patients treated with NAC.

Elnashar et al. showed that NAC is not an effective remedy to induce ovulation in CC-resistant PCOS patients[10], 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.[18] A recent study has found significant increase in ovulation, pregnancy rates and better reproductive outcome in PCOS patients who received NAC after unilateral laparoscopic ovarian drilling.[19]

Rizk et al. also 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 and pregnancy rate in women with CC resistant PCOS compared to the CC plus placebo group[20]. Our data support the results of their study, but based on our findings, NAC may be beneficial as an adjuvant to CC for induction of ovulation in a more expanded range of PCOS patients and not just limited to CC-resistant PCOS women.

No adverse effects of NAC were observed among PCOS patients in both studies, and the drug seemed to be safe and well-tolerated by all patients. It has been demonstrated that CC may have a negative 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.[21]

These adverse effects of CC on the endometrium may explain in part the relatively poor pregnancy rates associated with CC despite the high rate of ovulation[22]. In contrast to the study by Rizk et al., which did not reveal any significant change in endometrial thickness[20], 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.[23,24] 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

In conclusion, based on our data, NAC as an adjuvant to CC for induction of ovulation can improve the ovulation and pregnancy rates in PCOS patients and may also 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.

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