Original Article

N Acetyl Cysteine, A novel Remedy for Poly Cystic Ovarian Syndrome

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Abstract

Background: Poly cystic ovarian syndrome (PCOS) is the most prevalent endocrinopathy among women and the most common underlying diagnosis for anovulatory infertility. The role of insulin-resistance (IR) and hyperinsulinemia in pathophysiology and clinical manifestations of the syndrome depicts the importance of evaluation of the efficacy of insulin reducing medications. N acetyl cysteine (NAC) inhibits oxidative stress and prevents hyperglycemia induced insulin resistance. This study aims at evaluating the effects of NAC on manifestations of the disease as well as improvement of fertility status.

Materials and Methods: Through a prospective double-blind clinical trial, 46 patients were randomly divided into one intervention and one control group. The two groups were treated for six weeks after similarity was allocated. All clinical and biochemical indicators were recorded in the early follicular phase both before and after treatment.

Results: From each group, 18 patients were ultimately evaluated. In the first group, ovulation rate increased as compared to the control group. A significant decrease in weight, body mass index (BMI), and waist/hip ratio was also observed. Fast blood sugar (FBS), serum insulin, total cholesterol, low density lipoprotein (LDL) levels, and HOMA-IR index also dropped while high density lipoprotein HDL levels elevated significantly. No significant change was reported in luteinizing hormone (LH), FSH, PRL, LH/FSH levels and glucose/insulin ratio. The control group remained unchanged.

Conclusion: N- Acetyl Cysteine improves lipid profile, hormonal levels, ovulation status, and long-term health of women with PCOS. Considering its limited adverse effects, it can be regarded as a substitute for insulin reducing medications in treatment of these patients.

Keywords: Polycystic Ovarian Syndrome, Insulin Resistance, N Acetyl Cysteine

Introduction

Poly cystic ovarian syndrome (PCOS) is defined as an ovarian dysfunction syndrome in which a combination of heterogeneous symptoms and signs which manifests as a wide spectrum of the disorder (1). It is considered to be the most prevalent endocrinopathy resulting from anovulation and affects 5-10% of women (2-5). To a great extent, etiology of the syndrome has remained unknown although it has been revealed that synthesis of high levels of androgen and insulin-resistance (IR) lie at the core of its pathophysiology (6, 7). Sententious insulin secretion is observed in 70% of obese and 30% of thin patients (8-10). Chronic hyperinsulinemia results in different clinical pathologies such as diabetes mellitus- type II (DM- II), hypertension, and cardiovascular diseases (5, 11, 12). Risk factors of cardiovascular diseases tend to accumulate in these women even at their young age (5, 13),

and reports of dyslipidemia in these patients is correlated with insulin resistance (14). It has been proven that IR results in a disturbed response of glucose to insulin stimulation in skeletal muscles, and increase of hepatic glucose production as well as lipolysis (15-17). While post-receptor dysfunction in the pathway of insulin activity has been introduced as the reason for insulin resistance, the underlying reason for such a dysfunction still remains equivocal (18). On the other hand, insulin resistance and compensatory hyperinsulinemia accelerate the effect of luteinizing hormone (LH) on ovarian techa and androgen synthesis (19, 20) which inhibits ovulation through cessation of the follicular maturity process (21). As a consequence of unlimited exposure to estrogen following anovulation, these women are at a higher risk for endometrial cancer (22). There are 40% of women

suffering from PCOS that have to deal with infertility (23, 24) and abortion is the end result of pregnancies which occur in this group (23, 25-27). The underlying causes of infertility related to PCOS include oligo or anovulation, dysfunctional gonadotropin secretion, dysfunction of ovarian growth factors and proteins binding to them, high androgen levels, and insulin resistance (28). According to available data, evaluation of the effectiveness of insulin reducing medications in PCOS patients is an essential requirement (8, 29). Different studies have proven that treatment with insulin- sensitizing agents results in a decrease of plasma lipids, reduction of hyperandrogenism, regulation of menstrual cycles, and promotion of both spontaneous and induced ovulation (23, 30) whether the patient is clomiphen citrate (CC) resistant or not (31, 32). It is important to note that not all patients respond to these medications (33). Besides, such remedies might even increase the risk of concomitant metabolic or cardiovascular diseases (34). Moreover, these medications increase serum homocysteine (Hcy) levels in some patients (35-38). Hyperhomocysteinemia has been recognized as a risk factor for cardiovascular disease (CVD), early onset coronary, cerebral and peripheral atherosclerosis (39, 40), deep vein thrombosis, thrombo-ambolia (41, 42), pre-eclampsia, and recurrent abortion (28, 43-46). Studies have shown that taking N acetyl cysteine (NAC) reduces plasma Hcy levels (47-49). Through acceleration of glutation synthetase hormone (GSH) (50, 51) synthesis, an important antioxidant, further to inhibition of oxidative stress and consequently the prevention of hyperinsulinemia induced insulin resistance and preservation of insulin receptors against oxidant agents (8, 52, 53), NAC probably influences insulin receptor activity (8, 54) and results in an increase of glucose consumption which is an indicator of the insulin sensitivity state (55, 56). Fulghesu et al. have illustrated the fact that PCOS patients with hyperinsulinemia treatment with NAC results in improvement of insulin sensitivity, and a significant decline in plasma testosterone and lipid levels (8). Kilic-Okman and Kucuk have described NAC as an effective medication for the reduction in serum insulin and testosterone levels and improvement of Hcy status as well as lipid profiles among PCOS patients (48). Rizk et al. have noted that the combination of CC and NAC increases ovulation and pregnancy rates in CC-resistant PCOS patients who also suffer from infertility (57). In 2007, Badawy et al. noted that NAC would be effective in the induction and increase of ovulation and, as compared to placebo, the addition of NAC to a CC regimen in patients with PCOS would increase ovulation rates significantly (58). Also in 2007, Elnashar et al. showed that NAC per se would not be an effective remedy to induce ovulation in CC-resistant PCOS patients; in contrast to the significant decline in total testosterone levels, no significant change would be observed in post-treatment fasting glucose and insulin levels (59). In the present study, we have evaluated the effect of NAC on clinical, metabolic, hormonal, and fertility aspects of PCOS among patients.

Materials and Methods

Through a prospective experimental study designed as a clinical trial, in the interval between February 2007 to February 2008, 46 infertile patients diagnosed with PCOS who were referred to IVF Unit of the Reproductive Health Research Center of Shahid Beheshty University of Medical Sciences at Taleghani Training Hospital were enrolled. Inclusion criteria dictated that the patients needed to fulfill PCOS diagnostic criteria based on the Rotterdam consensus workshop in 2003 (60). That is, in order to be enrolled, each patient needed to have two out of three chronic oligo or anovulation criteria, clinical or biochemical hyperandrogenism or polycystic ovaries at sonography examination, spontaneous onset of maturation, normal sexual development and normal glomerular filtration rate (GFR). Presence of infertility factors other than anovulation, pelvic organic pathologies, congenital adrenal hyperplasia, thyroid dysfunction, Cushing's syndrome, hyperprolactinemia, androgen secreting neoplasia, diabetes mellitus, consumption of medications affecting carbohydrate metabolism and taking hormonal analogues other than progesterone two months prior to enrollment, severe hepatic or kidney diseases, and active peptic ulcer were considered as exclusion criteria.

Overall, considering a 95% confidence interval and 80% power, 46 patients were required as the sample population. Informed consent, which had been approved by the Research Ethics Committee was provided before enrollment. All patients were advised to avoid any changes in their physical activity and nutrition and not to undergo any new pharmacotherapy during the study. Patients' weight and height were measured so that the body mass index (BMI) could be calculated. Patients with BMI exceeding 30 were considered obese. The waist/hip ratio (WHR) was calculated by means of dividing waist size by hip size. WHR exceeding 85% was considered as high risk (61). Patients' blood pressures were also recorded. In order to avoid interference of menstrual cycle phases

with results, blood samples were provided for each individual in the early follicular phase, namely, on the third day of menstruation following spontaneous menstruation or menstruation induction by means of progesterone injection or withdrawal of oral medroxy-progesterone acetate. The samples were taken after eight hours of fasting and collected in sterile glass tubes. Serum was separated after blood samples were centrifuged and preserved in -20°C for the time of evaluation and analysis. All patients also underwent a basic sonography examination using a Honda Electronics HS 4000 (Japan), HSC-47 10 mv 5.0 MHZ 10 R microconvex transvaginal probe.

Patients were divided into two groups considering the similarity of compound confounding variables and randomized in order to minimize the effects of confounding factors through a randomized method. One group received treatment with NAC and the other, placebo. Medication was provided to patients by a midwife. Both the patient and physician were blinded to the type of treatment regimen provided. Treatment started on the third day of menstrual cycle in all patients. The experimental group received 1.8 g of NAC (N acetyl cysteine 600 mg, Holzkrichen, Germany, batch no. 6N5483) divided into three daily doses and the control group took placebo (ORS, Poursina, Tehran, Iran, batch no.30) with a similar method. Ovulation was monitored by regular ovulation cycles and by sonographic exams during the study. Patients were asked to report any possible adverse effects. At the end of treatment; weight, waist size, hip size, and blood pressure were measured for a second time in the early follicular phase. BMI and WHR were calculated and blood samples were provided. Total cholesterol levels (mg/dl) and LDL (mg/dl) were measured using photometry and the Friedewald formula, respectively. HDL (mg/dl) and triglyceride (TG) levels were measured with enzymatic colorimetry using a Pars Azmun Co. kit, Tehran, Iran. Follicle stimulating hormone (FSH) and luterine stimulating hormone (LH) (mIU/ml) were measured by immuno reactive multi analysis (IRMA; Izotop, Budapest, Hungary). Prolactin (PRL) (ng/ml) was also measured by Immuno reactine multi analysis (IRMA) (Izotop, Budapest, Hungary). Total testosterone levels (ng/ml) measured by enzyme immunoassay (EIA; Immunoassay, Izotop, Budapest, Hungary) were 0.2 to 1 among women. Fasting blood sugar (FBS; mg/dl) and fasting insulin (Mu/L) levels were measured by means of enzymatic colorimetry (Pars Azmun Co. Tehran, Iran) and EIMA (Mercodia, Uppsala, Sweden), respectively. Insulin resistance was evaluated through fasting insulin levels (>20 mU/L), glucose/insulin ratio (<4.5 mg/mU) and homeostatic model of assessment (HOMA). HOMA index (>2.5) which is calculated through the glucose × insulin/450. Data analysis was conducted by SPSS version 15.00. Comparison of the effects of NAC and placebo on patients with PCOS was performed by t test, and a comparison of the effect of NAC and placebo before and after treatment was conducted by paired t test. Correlation of quantitative parameters was calculated by Pierson correlation co-efficient and comparison of qualitative parameters before and after treatment with NAC and placebo was conducted by Chi square. A p value beneath 0.05 was considered statistically significant.

Results

A number of patients in each of the two groups (experimental and control) dropped out. One patient in the experimental group withdrew consent as a result of intolerance to the medication's odor In addition, some blood samples were either not theoretically or practically appropriate for the study. Finally, evaluations were limited to 18 patients in each of the two groups. The experimental and control groups were homogeneous considering their demographic and basic characteristics (Table 1) such as: age, duration of marriage, duration of infertility, family history, hirsutism, acne, and alopecia. Homogeneity was also observed as their clinical status (Table 2) was taken into consideration: namely weight, height, waist size, hip size, WHR, systolic and diastolic blood pressure, metabolic, endocrine and basic hormonal (Table 3) parameters including FPG, fasting insulin, total cholesterol, LDL, HDL, TG, LH, FSH, PRL, and testosterone (TT) as well as the respective ratios such as glucose/insulin, HOMA-IR index, and FSH/LH. No statistically significant difference was observed in any of the aforementioned parameters between the two groups. After completion of the treatment course, no significant change was observed in the results of sonographic examinations, improvement of acne, hirsutism, and alopecia. No significant change in diastolic and systolic blood pressure was observed, either. Ovulation rate following consumption of NAC was reported to be 55.60% (10.18) as compared to 16.7% (3.18) in the control group (p= 0.035). The six week treatment course with NAC resulted in significant changes in weight and consequently BMI of the patients as well as in their waist and hip size. As a result, a significant change was also observed in WHR (Table 2).

Table 1:Clinical characteristics of the two groups of patients

	NAC receiving group	Placebo receiving group	P value
Number of patients (%)	50 (18)	50 (18)	
Mean age (SD)	27.22 (5.35)	27.89 (6.10)	0.730
Mean duration of marriage (SD)	6.67 (4.13)	6.06(4.39)	0.670
Mean duration of infertility (SD)	4.50 (2.17)	4.22(3.28)	0.767
Number of amenorrhea patients (%)	2 (11.1)	2 (11.1)	NS
Number of patients with positive family history (%)	3 (16.7)	4 (22.2)	NS
Number of hirsute patients (%)	11 (61.1)	10 (55.6)	NS
Number of patients with acne (%)	5 (27.8)	5 (27.8)	NS
Number of patients with alopecia (%)	3 (16.7)	1(5.6)	NS

Table 2: Clinical features before and after treatment with N Acetyl Cysteine or placebo in PCOS patients

	Before NAC treatment	Before placebo treatment	P value	After NAC treatment	After placebo treatment	P value (diff)
Weight (Kg)	74.14 (11.67)	74.14 (13.24)	1.000	72.25 (11.91)	74.16(12.98)	<0.001
Height(m)	158.39 (7.00)	158.44(4.61)	0.978	158.39 (7.00)	158.44 (4.61)	NS
BMI (kg/m²)	29.50 (4.12)	29.45 (4.38)	0.967	28.75 (4.24)	29.347 (4.35)	<0.001
Waist size (cm)	85.67 (11.29)	84.50 (11.49)	0.759	83.72 (11.49)	84.61 (11.20)	0.001
Hip size (cm)	108.28 (9.27)	107.44 (9.23)	0.789	107.44 (9.55)	107.44 (9.23)	0.014
WHR	0.78 (0.05)	0.78 (0.04)	0.768	0. 77 (0.05)	0.78 (0.04)	0.006
SBP* (mmHg)	114.44 (9.37)	113.33 (6.64)	0.684	112.77 (7.51)	113.33 (6.85)	0.401
DBP (mmHg)**	79.44 (13.16)	74.72 (8.48)	0.209		79.44 (13.16)	0.059

* System blood pressure, ** Diastolic blood pressure
( ): SD

Table 3: Metabolic, endocrine and hormonal parameters before and after treatment with N Acetyl Cysteine or placebo in women with PCOS

	Before NAC treatment	Before placebo treatment	P value	After NAC treatment	After Placebo treatment	P value (diff)
FPG (mg/dl)	95.55 (10.91)	96.83 (27.70)	0.857	8606 (8.97)	101.34 (29.76)	< 0.001
Fasting insulin (mU/L)	23.93(19.73)	19.48 (15.82)	0.461	20.88(18.14)	22.15(19.7)	0.005
Glucose/ insulin	6.03 (4.56)	7.43 (4.39)	0.356	7.25(8.46)	10.96(17.99)	0.576
HOMA-IR	5.22(5.58)	4.90 (4.2)	0.553	4.67(4.56)	5.94(6.09)	0.001
Chol(mg/dl)	175.17 (25.12)	176.50 (31.85)	0.890	162.38 (26.60)	176.88 (31.55)	0.009
HDL(mg/dl)	43.59(12.08)	44.42(10.56)	0.828	45.33 (14.16)	42.83(11.03)	0.012
LDL(mg/dl)	105.96(21.74)	176.50 (31.85)	0.899	93.40 (17.38)	108.53 (28.97)	<0.001
TG(mg/dl)	146.72 (58.91)	112.00(65.75)	0.104	122.72(54.31)	115.83(57.32)	0.001
LH(mIU/ml)	6.74 (2.80)	1.18 (0.52)	0.694	6.70 (2.70)	6.79 (3.15)	0.514
FSH(mIU/ml)	6.45 (1.69)	6.00 (1.86)	0.452	9.73 (1390)	5.87 (1.78)	0.290
LH/FSH	1.08 (0.46)	1.18 (0.52)	0.573	0.95 (0.38)	1.13 (0.41)	0.473
PRL(ng/ml)	5.91 (3.35)	8.44 (4.99)	0.084	6.20 (4.41)	11.50 (13.47)	0.378
TT (ng/ml)	0.91 (0.48)	1.01 (0.45)	0.537	0.76 (0.42)	1.00 (0. 43)	0.011

( ): SD

Serum FPG, fasting insulin, total cholesterol, LDL, TG, and TT levels dropped significantly while serum HDL levels showed a significant rise (Table3). Nevertheless, no significant change was observed in serum LH, FSH, and PRL levels, and LH/FSH ratio (Table 3). While an improvement in the glucose/insulin ratio was noted, such a change was not statistically significant. At the same time, the HOMA-IR index depicted a significant drop after treatment with the NAC regimen. Overall, insulin resistance which was determined by a combination of fasting insulin, glucose/insulin ratio and HOMA (HOMA-IR) index had improved after NAC treatment. In the placebo group, the changes were neither significant nor improvement of the patients' PCOS status was noted.

Discussion

In PCOS; IR or compensatory hyperinsulinemia, hyperandrogenemia, disrupted foliculogenesis and dyslipidemia are distinct manifestations which are somehow correlated to each other and increase the risk of cardiovascular diseases, diabetes mellitus, hypertension, thrombosis, infertility and endometrial cancer in patients.

This study has been conducted to evaluate the therapeutic effects of NAC on clinical, metabolic, hormonal, and fertility aspects of the disease as well as a comparison of the therapeutic effects with placebo in women suffering from PCOS. The results illustrate the fact that after six weeks of treatment with NAC, patients showed a significant drop in weight, BMI, waist size, hip size, and WHR which has been recognized as the strongest predictor of insulin resistance (55-58). In a study conducted by Fulghsu, 31 out of 37 women who were enrolled were obese. In this study, the administration of NAC did not result in any significant change in BMI (8). In the study conducted by Elnashar on 64 women suffering from CC- resistant PCOS, patients were divided to two groups;, one receiving NAC and the second Metformin for a period of six weeks. Here again, no significant change was reported in either BMI or WHR (53). Studies on other insulin sensitizing agents such as Thiazolidions and Pioglythazone have reported weight gain in patients (59, 60).

About 30-60% of women with PCOS are overweight and obese (61). There are 70% of obese women with PCOS who have exaggerated insulin secretion. When combined with the synergic effect of insulin resistance resulting from the presence of excessive fat and hyperinsulinemia that is caused by the syndrome (8-10), and since all manifestations of the syndrome are somehow correlated to

IR and hyperinsulinemia (23); one can conclude that reduction of the lipid mass following NAC consumption plays a manifest role in improvement of PCOS patients.

Besides, our study revealed that NAC is effective in the improvement of lipid profile through causing significant decrease in total cholesterol, LDL, TG, and a significant rise in HDL. This result is concordant with the results of previous studies (48, 56).

In contrast to the significant drop in serum fasting plasma glucose (FPG) and fasting insulin levels, the rise in glucose/ insulin ratio was not found to be statistically significant. HOMA-IR index also showed a decline. Thus, it seems that NAC consumption has been accompanied with an improvement in insulin sensitivity and glucose utilization. Treating 20 PCOS women with NAC, Kilic noticed that despite the insignificant change in fasting plasma glucuose (FPG) (p=0.20), insulin levels dropped significantly (p=0.001) and the decline in HOMA-IR index was also statistically significant (p= 0.001). In a study conducted by Fulghesu through which insulin sensitivity was assessed using the colompe euglycemic hyperinsulinemic technique, patients with PCOS showed an increased sensitivity to insulin and a significant decline in insulin area under curve (AUC) after oral glucose tolerance test (OGTT) while FPG, fasting insulin, and glucose AUC remained intact (8). Likewise, Elnashar reported that FPG and serum insulin levels dropped significantly among patients who received Metformin while such a change was not significant in the group of patients being treated with NAC. In his study, the glucose/ insulin ratio did not show a significant change in the two groups (59).

In our evaluations, a significant decline in post-treatment TT was observed. There were similar results reported by previous studies in this regard (8, 48, 53).

It has been proposed that there is a correlation among hyperinsulinemia, IR, and ovarian hyperandrogenism among PCOS patients (59). On one hand, testosterone induces insulin resistance in these patients' cells (59) and on the other hand, insulin resistance and compensatory hyperinsulinemia accelerate the effect of LH on ovarian theca cells and androgen synthesis is promoted (15, 16, 55, 59). In fact, NAC causes a decline in the androgenic response of the ovary to gonadotropin stimulation.

After a six week NAC treatment course was completed, ovulation rate was 55.60% in the experimental group versus 16.70% among the control

group. This is indicative of the fertilizing effects of the medication in PCOS patients. Through a five day treatment of obese CC- resistant PCOS patients with NAC, Rizk has also reported a significant increase in ovulation rate (49.3%) and pregnancy rate (21.3%) as compared to the control group (1.3% and 0%; p< 0.0001, p=0.00006) (62). Meanwhile, after a six week treatment course, Elnashar reported the ovulation rate to be 6.7%(2.30) in the group receiving NAC as compared to 51.68%(16.31) among patients treated by Metformin. He concluded that NAC per se can not be considered an effective medication in ovulation induction among women with CC- resistant PCOS (53).

Hyperandrogenism interferes with follicular maturity in women with PCOS and results in anovulation (17). It seems that to further ovulation induction, NAC preserves more follicles in the ovary through its anti-apoptotic mechanism (62). But in our study, no change was observed in sonographic characteristics of the ovaries after treatment.

Contrary to the introduction of Metformin as the most common insulin reducing medication resulting in improvement of insulin sensitivity, reduction of serum insulin and free testosterone levels (38, 39), various studies have concluded that Metformin does not result in an improvement of a hyper-insulinemic and excessive testosterone state (39). Recently, one randomized clinical trial (RCT) has proven that patients who take placebo in combination with CC have no significant difference in ovulation and pregnancy rates when compared with PCOS patients taking Metformin together with CC (59, 15). But the heterogeneity of the groups being studied and the differences in assessment methods as well as duration of treatment (53) should be taken into consideration; it seems that the beneficial effects of Metformin are manifest in some special groups (55). Assessment of such an issue in comparison to the therapeutic effects of NAC in different subgroups of patients considering age, BMI, WHR, serum insulin and androgen levels and induced menstrual cycles should be considered in future studies.

Overall, as compared to the long course of treatment (12- 24 week) with other insulin- reducing medications, the highly significant changes brought by a six week course of treatment with NAC suggest the hypothesis that longer treatment with NAC may result in more desirable outcomes, such as more effective control of clinical hyperandrogenism. An assessment of the therapeutic effects of the medication when combined with behavioral modification can also be considered for future studies.

So far, no adverse effects have been reported for NAC (8, 53, 62). It seemed to be well- tolerated by our sample population. Only one patient with a history of mental disorder refused to cooperate because of intolerance to the medication odor. Meanwhile, the high adverse profile of Metformin which results in a high percentage of drop-outs must be noted (15, 55). Rare serious side effects have also been reported from Metformin and thiazolidions (61). Besides, these medications increase serum homocystein (Hcy) levels (46) while various studies have reported that NAC induces a drop in serum Hcy levels (48, 49).

Conclusion

It can be concluded from the results of the present study that NAC promotes lipid profile, hormonal levels, ovulation, and consequently the long-term health status of women with PCOS through inhibition of oxidative stress and improvement of peripheral insulin. Taking its lack of adverse effects into consideration, NAC can be regarded as an appropriate substitute for insulin- reducing medications in the treatment of PCOS patients.

Acknowledgements

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