Article

N-acetyl cysteine for treatment of recurrent unexplained pregnancy loss

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Abstract

Pregnancy could be associated with a state of oxidative stress that could initiate and propagate a cascade of changes that may lead to pregnancy wastage. This process of oxidative stress may be suppressed by the antioxidant effect of *N*-acetyl cysteine (NAC). The current study aimed to evaluate the effect of NAC therapy in patients diagnosed with unexplained recurrent pregnancy loss (RPL). The study was a prospective controlled study performed in the Women's Health Centre, Assiut University, Egypt. A group of 80 patients with history of recurrent unexplained pregnancy loss were treated with NAC 0.6 g + folic acid 500 µg/day and compared with an aged-matched group of 86 patients treated with folic acid 500 µg/day alone. NAC + folic acid compared with folic acid alone caused a significantly increased rate of continuation of a living pregnancy up to and beyond 20 weeks [$P < 0.002$, relative risk (RR) 2.9, 95% confidence interval (CI) 1.5–5.6]. NAC + folic acid was associated with a significant increase in the take-home baby rate as compared with folic acid alone ($P < 0.047$, RR 1.98, 95% CI 1.3–4.0). In conclusion, NAC is a well-tolerated drug that could be a potentially effective treatment in patients with unexplained RPL.

Keywords: *antioxidant, folic acid,* N*-acetyl-cysteine, pregnancy, recurrent miscarriage*

Introduction

Recurrent pregnancy loss (RPL) is defined as the occurrence of three or more consecutive pregnancy losses in the first or early second trimester of pregnancy (less than 20 weeks of gestation). It is one of the most common clinical problems in reproduction, yet a definite cause can be established in only 50%% of cases [American College of Obstetricians and Gynecologists (ACOG, 2002)]. Many aetiologies have been suggested for RPL and evidence has been accumulating that oxidative stress may be a contributory factor (Poston and Raijmakers, 2004). Oxidative stress occurs when the generation of free radicals (i.e. substances with one or more unpaired electrons) exceeds the capacity of the antioxidant defence mechanisms (i.e. pathways that provide protection against the harmful effects of free radicals). Free radicals induce damage of cells, lipids and proteins (Halliwell, 1996).

Pregnancy has been viewed as a state of oxidative stress that results from increased placental mitochondrial activity and production of reactive oxygen species (ROS), mainly superoxide anions and nitric oxide which have pronounced effects on placental function including trophoblast proliferation and vascular activity (Hempstock *et al*., 2003; Jauniaux *et al*., 2003a; Myatt and Cui, 2004). Excessive ROS production may occur in the first trimester when establishment of blood flow into the intervillous space is associated with a burst of oxidative stress (Jauniaux *et al*., 2000). In addition, it has been shown that ROS in IVF culture media may affect post-fertilization development of embryos (Agarwal *et al*., 2005). The inability to mount an effective antioxidant defence against this may be a contributory factor in early pregnancy loss (Jauniaux *et al*., 2003b).

In-vitro studies have shown that oxidative stress might serve as a signal to initiate and propagate the inflammatory process and result in apoptosis of placental tissues. Placental oxidative

stress may represent a common pathophysiological pathway for different aetiologies of RPL (Jauniaux and Burton, 2005). This process could be suppressed by the antioxidant effect of *N*-acetyl cysteine (NAC) (Ohyama *et al.*, 2001). In addition, NAC may have a role in the prevention of lipopolysaccharide (LPS)-induced fetal developmental toxicity and thus preventing the negative effects of LPS that may induce intrauterine fetal death and intrauterine growth retardation (Xu *et al.*, 2005).

NAC is a safe and well-tolerated mucolytic drug that softens tenacious mucous secretions. It is the acetylated precursor of both amino acid L-cysteine and reduced glutathione. Experimental animal studies have shown that NAC has a protective effect against methyl mercury embryotoxicity in mice (Ornaghi *et al.*, 1993). Additionally, it prevents the teratogenic effect of diabetic serum in rat embryo culture (Wentzel *et al.*, 1997). In humans, although NAC treatment was not successful in stabilizing pre-eclamptic toxaemia in a randomized controlled trial, the study showed no maternal or fetal harmful effects of NAC treatment (Roes *et al.*, 2006). Moreover, its implications for human reproduction were recently highlighted in a study that showed potential benefits of NAC co-treatment in patients with chronic anovulation (Rizk *et al.*, 2005). As far as is known, the potential use of NAC in the management of patients with unexplained RPL has never been tested. The current study aimed to compare the effect of NAC + folic acid supplementation versus the use of folic acid alone on the outcome of pregnancy in patients with unexplained RPL.

Materials and methods

This was a hospital-based prospective controlled study conducted in the Women's Health Centre, Assiut University Hospital, Assiut, Egypt in the period from May 2005 to May 2007. Eligible participants were between 20 and 35 years of age with a history of RPL with the same partner. All patients were regularly menstruating before the current pregnancy and conceived spontaneously. Patients with consanguineous marriage, uterine anatomic abnormalities, those with positive antibodies for antiphospholipid syndrome [anticardiolipin (immunoglobulin G and immunoglobulin M) antibodies and anti-lupus anticoagulant] and those suffering from any endocrine abnormalities were excluded from participation. In addition, those who received any hormonal, antioxidant medications or folic acid within 3 months before the current pregnancy were also excluded from study. All participants signed a written consent after reading the patient information sheet or after having it read to them. The Assiut University Medical School Ethical Review Board approved the study.

A group of 80 eligible pregnant women were prospectively followed up during and after receiving of 0.6 g NAC (Sedico, Cairo, Egypt) per day orally in the form of sachets plus folic acid 500 µg (tablets) per day (group 1). Another 86 eligible participants with comparable demographic criteria and obstetric history were recruited during the same time period during and after treatment with only folic acid 500 µg (tablets) per day (group 2). Patients started treatment once pregnancy was diagnosed (urinary human chorionic gonadotrophin and transvaginal ultrasonography) (usually day 35 post-menstrual). All participants were followed up clinically and by ultrasound scan every 2 weeks, up to week 20 of gestation. Afterwards, patients underwent the routine antenatal follow-up schedule by the same investigators. Patients were asked to report any side effects of treatment and any complaints as regards their pregnancies. Fetal viability and growth were monitored by ultrasound scan. The treatment continued up to week 20 of gestation or proved occurrence of miscarriage by ultrasound scan.

The primary outcome measure was continuation of a viable pregnancy beyond week 20 of gestation. Secondary outcome measures were also assessed including the side effects of treatment, the rate of fetal growth, occurrence of congenital malformations, amniotic fluid volume, continuation of pregnancy to viability/term, and the take-home baby rate.

Sample size calculation was based on the primary outcome (continuation of pregnancy beyond 20 weeks). Previous studies reported that 35% of couples with unexplained recurrent pregnancy loss will eventually have a successful pregnancy if they do not receive treatment or take a placebo (ACOG, 2002). Using two sided chi-squared test with α of 0.05, a total sample size of at least 160 in two groups will have 85% power to detect a 25% difference in the proportion of successful pregnancies between the two study groups (i.e. 60% in the treatment group versus 35% in the control group) (odds ratio of 2.7) assuming a loss to follow up rate of 10% (nQuery Advisor 4.0, Statistic Solutions, Saugus, MA, USA).

The data were entered on a Microsoft Access database and analysed anonymously using the Statistical Package for Social Science (SPSS Inc., Chicago, version 13, USA). Comparisons between the groups were performed using Student's *t*-test to compare the mean values between groups in scale variables. However, chi-squared test was used to compare the dichotomous and ordinal variables in the groups. For analysis, $P < 0.05$ was considered significant.

Results

A total of 168 patients were recruited in two groups: group 1 received NAC and folic acid ($n = 80$) and group 2 received folic acid only ($n = 86$). **Table 1** shows that there were no statistically significant differences between the two study groups regarding age, previous obstetric history and the percentage lost to follow-up.

Table 2 shows that the rate of successful pregnancy continuation up to week 20 was significantly higher in group 1 treated with NAC and folic acid [$P < 0.002$, relative risk (RR) 2.9, 95% CI 1.5–5.6] in comparison with group 2. Moreover, the incidence of take-home baby is significantly higher in the NAC + folic acid group compared with the folic acid only group ($P < 0.047$, RR 1.98, 95% CI 1.3–4.0). However, some patients in both groups reported vaginal bleeding (spotting) with no statistically significant difference between the two groups. These cases were evaluated by ultrasonography that showed no gestational abnormalities. No additional treatment was prescribed, patients were reassured and pregnancy continued in all these cases. Ten patients in the NAC + folic acid treatment group complained of minor gastrointestinal disturbances compared with only five patients in the group treated with folic acid alone; the difference was not statistically significant. In addition, ten patients (12.5%) in group 1 reported some inconvenience of NAC, due to its mucolytic effect.

Table 3 shows the discontinuation of pregnancies in the two groups. Statistically significantly more pregnancies discontinued

Table 1. Characteristics of the two study groups (women with recurrent unexplained pregnancy loss treated with *N*-acetyl cysteine + folic acid or folic acid alone).

Characteristic	*Treatment*	
	N-acetyl cysteine + folic acid	*Folic acid*
No. recruited	80	86
No. followed up to 20 weeks (%)	78 (97.5)	86 (100)
No. followed up to end of pregnancy	78 (97.5)	82 (95.3)
Age (years)	26.2 ± 3.74 (16–35)	25.19 ± 3.95 (19–33)
Mean parity	0.7 ± 0.877 (0–3)	0.73 ± 0.931 (0–3)
No. of previous miscarriages	3.05 ± 1.14 (3–7)	3.98 ± 1.124 (3–7)
No. of previous preterm labours	0.11 ± 0.356 (0–2)	0.13 ± 0.333 (0–1)

Values are mean ± SD (range) unless otherwise stated.
There were no statistically significant differences between the two groups.

Table 2. Outcomes for women with recurrent unexplained pregnancy loss treated with *N*-acetyl cysteine + folic acid ($n = 80$) or folic acid alone ($n = 86$).

Outcome	*Treatment*		P-*value*[a]	*Relative risk (95% confidence interval)*
	N-*acetyl cysteine + folic acid*	*Folic acid*		
Viable pregnancy to 20 weeks	58 (72.5)	44 (51.2)	0.002	2.90 (1.5–5.6)
Live birth	45 (56.3)	32 (37.2)	0.047	1.98 (1.3–4.0)
Intrauterine growth retardation	16 (20.0)	12 (14.0)	NS	–
Oligohydramnios	4 (5.0)	7 (8.1)	NS	–
Gastrointestinal disturbance	10 (12.5)	5 (5.8)	NS	–

Values are number (percentage). NS = not statistically significant.
[a]Chi-squared test.

Table 3. Continuation of pregnancies in women treated with *N*-acetyl cysteine + folic acid compared with those treated with folic acid alone.

Duration of pregnancy	*Treatment*[a]	
	N-*acetyl cysteine + folic acid*	*Folic acid alone*
<10 weeks	11 (13.8)	25 (29.1)
10–19 weeks	9 (11.3)	16 (18.6)
20–27 weeks	1 (1.3)	9 (10.5)
Preterm delivery (≥28 weeks)	11 (13.8)	9(10.5)
Term delivery	46 (57.5)	23 (26.7)
Lost to follow-up	2 (2.5)	4 (4.7)

Values are number (percentage).
[a]The rate of pregnancy loss rate was statistically significantly different between the two treatment groups ($P < 0.0003$).

in the folic acid alone group in comparison with NAC + folic acid group ($P = 0.0004$).

Discussion

This study reports on the use of NAC for the treatment of unexplained RPL in a large cohort of patients. NAC therapy was associated with significant prolongation of gestation in patients with a history of unexplained RPL. Miscarriage and pregnancy have been associated with a variety of biological phenomena including increased oxidative stress, angiogenesis and apoptosis. In a successful pregnancy, however, changes occur within the peripheral blood that offer protection from the negative effects of free radicals (Jenkins *et al*., 2000). It has been shown that oxidative stress is associated with glutathione depletion and damage of the fetus (Buhimschi *et al*., 2003). In addition, it may trigger apoptosis, the consequences of which could be counteracted by the antioxidant properties of NAC (Jauniaux *et al*., 2003b). It is also likely that lower expression of angiogenesis-related and apoptosis-related genes is associated with RPL (Choi *et al*., 2003).

NAC is an excellent source of sulphydryl groups. It is a thiol-containing antioxidant that either increases intracellular glutathione concentrations (endogenous reducing agent) and or acts directly as a free radical scavenger (Cotgreave, 1997; Kelly, 1998). NAC was found to prevent apoptosis and oxygen-related genotoxicity in endothelial cells (Aluigi *et al*., 2000) by maintaining intracellular glutathione concentrations and reducing mitochondrial membrane depolarization (Quadrilatero and Hoffman-Goetz, 2004). It suppresses nuclear factor DNA-binding activity and resultant gene expression. It also inhibits phospholipid metabolism and pro-inflammatory cytokine release (Lappas *et al*., 2003). Consequently, NAC could modify oxidative stress, angiogenesis and apoptosis-triggered reactions in normal and abnormal pregnancy. In the present study, it was found that administration of NAC + folic acid to patients with unexplained RPL significantly increased pregnancy viability to 20 weeks of pregnancy ($P < 0.002$). This positive effect was reflected by a higher take-home baby rate in the group of patients treated with NAC. This favourable response to NAC could be explained by the aforementioned biological effects.

NAC is a safe and well-tolerated drug during pregnancy and has been previously used for treatment of acute acetaminophene toxicity during pregnancy (Riggs *et al*., 1989). The side effects reported in this study were mild and did not necessitate discontinuation of treatment. The results of this study are encouraging; however, one of the shortcomings is not having performed routine karyotyping to meet the ACOG criteria for recurrent unexplained pregnancy loss. For this reason, women with increased maternal age and those with consanguineous marriage were excluded from the study. A larger randomized clinical trial is recommended to evaluate the efficacy and long-term outcome. Also evaluation of indicators of oxidative stress and apoptosis in placental tissue of those who responded to treatment and those who miscarried is recommended.

In conclusion, NAC could potentially be clinically useful in the management of unexplained RPL. It is a simple, well-tolerated and inexpensive agent.

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