Effect of N-Acetylcystiene on maternal serum interleukin-8 in pregnant women with History of Idiopathic Preterm Labor

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Abstract: Background: Oxidative stress was proposed to play a role in pathophysiology of prematurity. N-acetylcysteine had been hypothesized to have preventive role on prematurity. **Aim of the work:** to study the effect of N-acetylcystiene in prevention of idiopathic preterm labor. **Patients and methods:** it was an clinical longitudinal prospective observational study conducted in Obstetrics & Gynecology department, Al-Azhar University hospital, New Damietta at the period from January to December 2015. It included 50 Pregnant women with previous history of idiopathic preterm labor. All included Pregnant women were submitted to: 1) Careful history taking, clinical examination and investigations. IL-8 was measured before administration of N-acetylcystiene. Then, each patient received daily dose of 0.6 g of NAC in oral effervescent from. The duration of NAC administration extended from 24 to 36 weeks' gestation. All females were followed up for nausea, vomiting, and other side effects of (NAC), after the end of treatment course, we take another blood sample at 36th weeks' gestation to measure IL-8 after N-acetylcystiene administration. Finally, all pregnant women followed up to labor and pregnancy outcomes were documented. **Results:** Five Pregnant women (10%) discontinued taking NAC owing to nausea, vomiting, headache and low blood pressure, 45 pregnant women which continued taking NAC, in whom there were a highly statistical significant deference between Value of IL-8 before NAC administration at 24 weeks with after 36 weeks when (p-value was <0.001) and mean±SD 0.89±0.66 At 24 weeks while At 36 weeks after NAC administration was 0.39±0.56. **Conclusion:** Using N-acetylcysteine in preventing preterm labor by affection on IL-8 as a pro-inflammatory cytokine could potentially be clinically useful in the management of preterm labor. As it is a simple, well-tolerated and inexpensive agent.

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1. Introduction

Premature birth is now the biggest global killer of young children, with more than 1 million children dying each year due to the complications of preterm birth, mostly in the developing world **(WHO, 2014).**

Preterm birth, defined as delivery before 37 weeks of gestation, has a global prevalence of 9.6 %. It is the leading cause of neonatal morbidity and mortality and is responsible for approximately 70 % of all neonatal deaths and 40 % of childhood neurological morbidities. **(Fernando et al., 2015)**.

Preterm birth accounts from 5–18% of all live births worldwide and is a significant contributor to infant mortality and long-term morbidity including an increased risk of severe impaired neurodevelopment and respiratory complications **(Blencowe et al., 2012).**

Oxidative stress, which is associated with pregnancy and infection, activates nuclear factor alpha, and interleukins-6 and interleukins-8. This leads to increased prostaglandin and cyclooxygenase-2 synthesis and induces the expression of matrix metalloproteinase P, which is implicated in preterm labor (**Amin et al., 2008)**.

Interleukin-8 (IL-8) belongs to the class of pro inflammatory chemokines defined by the position of two cysteine groups and is synthesized predominantly by monocytes. Its active form effectively activates neutrophil granulocytes, advancing the chemotaxis and synthesis of myeloperoxidase, thus suggesting a critical role in host defense to infectious diseases **(Attaurrahman et al., 2010).**

The presence of increased concentration of IL-8 in a variety of biological fluids including maternal and/or fetal blood, amniotic fluid, urine, cervical and/or vaginal secretions and placental tissue is an independent risk factor for preterm labor **(Hashemi and Shahshahan, 2014).**

There is a growing interest in therapeutic interventions target inflammatory labor cascade by blocking the production of pro-inflammatory mediators or up-regulating anti-inflammatory mediators and/or the exogenous administration of anti-

inflammatory or pro-resolution mediators **(Ng et al., 2015).**

There is a relationship between increased risk of spontaneous preterm delivery and high IL-8 levels **(Tosun et al., 2010; Rode et al., 2012; Cemgil Arikan et al., 2012).** However, there is no conclusive data on the early pregnancy cytokine levels and birth outcomes **(Elksne et al., 2013).**

N-acetylcysteine (NAC) is an acetylated cysteine residue, an optimal state has been demonstrated to be of primary importance if attempting to optimize the protective ability of the cell to oxidative stress **(Kerksick and Willoughby, 2005)**.

The present study was designed to investigate the effect of N-acetylcystiene in prevention of preterm labor by decreasing IL-8 as pro-inflammatory cytokine.

2. Patients and methods

This clinical longitudinal prospective observational study was conducted in Obstetrics and Gynecology department, Al-Azhar University hospital (New Damietta) at the period from January to December 2015. It included 50 pregnant women, with previous history of idiopathic preterm labor in the gestational age between 24 to 36 weeks.

Inclusion criteria:

All pregnant women between 24th and 36th weeks of pregnancy who had history of one or more spontaneous preterm labor of a live born singleton fetus.

Exclusion criteria:

pregnant women who had one or more of the following were excluded from the study: 1) Multiple pregnancy; 2) Threatened abortion in the current pregnancy; 3) Rupture of membranes; 4) History of incompetent cervical os; 5) Cerclage performed in previous or current pregnancy; 6) Recognized risk factors for preterm labor as bacterial vaginosis (BV) in the current pregnancy; 7) Intrauterine fetal death, polyhydramnios and fibroid uterus; and 8) Hypertensive disorder or any health problem possibly ends with iatrogenic preterm labor.

All included pregnant women were submitted to: 1) Careful history taking (personal- present- past) to check for the inclusion and exclusion criteria and Investigations (complete blood picture, random blood sugar, ABO grouping, RH typing and urine analysis, ultrasound examination).

Explanation of the study aim for each one of patients were done and an informed consent for participation in the study was obtained before completing a written sheet including (patient name, age, gravidity, parity, number of full term labor, number of preterm labor also body mass index was calculated.

In addition, to routine investigations, we collect a 1ml blood sample from each patient at booking at 24th week of gestation. Samples were collected into a serum separator tube. Serum was separated and stored at -20°C till the time of analysis.

We measure IL-8 by ELIZA kits before administration of N-acetylcystiene. Then, each patient received a daily dose of 0.6 g of NAC in oral effervescent from (Acetylcystiene, Sedico, Egypt). The duration of NAC administration extended from 24 to 36 weeks' gestation (12 weeks). All pregnant women were followed up for nausea, vomiting, and other side effects. After the end of treatment course, we take another blood sample (1 cm) from all pregnant women at 36th weeks' gestation to measure IL-8 after N-acetylcystiene administration. Finally, all pregnant women followed up to labor and pregnancy outcomes were documented.

Statistical methods:

Statistical presentation and analysis was conducted using the IBM, SPSS version 20 (SPSS Inc, USA, Chicago). Numerical data were presented as arithmetic mean and standard deviations. In addition, minimum (min) and maximum (max) values were presented. Categorical data were presented as relative frequency and percent distribution. For comparison between two means, independent samples (t) test was used. P value < 0.05 was considered significant.

3. Results

In the present study, 50 pregnant females were included. Their age ranged between 19-35 years (mean ±SD; 26.34±4.12), gravidity ranged between 2-7 gravid (mean± SD; 3.92±1.16), parity ranged between 1-6 Para (mean ±SD; 2.88±1.17), their weight range between 60-117 kg (mean ±SD; 380.28±15.58) and with previous history of preterm labor ranged between 1-5 preterm labors (mean ±SD; 1.78±1.0) (Table 1). There was a highly statistical significant deference between Value of IL-8 before NAC administration at 24 weeks with after 36 weeks when (p-value was <0.001) and mean ±SD At 24 weeks was 0.89±0.66 while At 36 weeks after NAC administration was 0.39±0.56 (table 1).

In the present study, five patients (10%) discontinued taking NAC owing to nausea, vomiting, headache and low blood pressure. Thirteen patients (26.0%) continued NAC and tolerated side effects while, thirty-two patients continued without reported complaints (table 2). At 24 weeks of gestation IL-8 value among pregnant females whose infants were admitted to incubator was significantly lower than those whose infants were not admitted to incubator (P-value <0.05). However, at 36 weeks of gestation IL-8 value among pregnant females whose infants were admitted to incubator was significantly higher than

those whose infants were not admitted to incubator (P-value <0.05) (table 3).

Table (1): demographic and laboratory data of studied females

Variable	Mean ±SD	Min-Max (range)
Age (years)	26.34±4.12	19-35
Body weight (kg)	80.28±15.58	60-117
Body mass index (kg/m^2)	30.07±4.61	24-45
Gravidity	3.92±1.16	2-7
Parity	2.88±1.17	1-6
Previous full-term labors	1.14±0.78	0-3
Previous pre-term labors	1.78±1.0	1-5
IL-8 at 24 weeks' gestation (n=50)	0.89±0.66	0.010- 2.25
IL-8 at 36 weeks gestation (n=45)	0.44±0.56	0.00 – 1.80

NB: Paired (t) was 4.41, p < 0.001* when compared IL-8 at 24 weeks to IL-8 at 36 weeks.

Table (2): Side effects of NAC administration

Variable	Statistics
Discontinued treatment	5/50 (10.0%)
Nausea	2/5 (40.0%)
Vomiting	2/5 (40.0%)
Headache &Low blood pressure	1/5 (20.0%)
Continued treatment and tolerated side effects	13/50 (26.0%)
Nausea	6/13(46.1%)
Vomiting	4/13(30.8%)
Headache	2/13(15.4%)
Low blood pressure	1/13(7.7%)
Continued with no complaints	32/50(64%)

Table (3): Relation between value of IL-8 before (24 weeks) and after NAC administration (36 weeks) and neonatal incubation

parameter	Baby incubation		Baby not-incubated		t	P value
IL-8	Mean	SD	Mean	SD		
At 24 weeks' gestations	0.966	0.646	0.182	0.072	2.69	0.010*
At 36 weeks' gestation	0.398	0.528	1.385	0.145	2.60	0.012*

4. Discussion

Preterm birth is defined as delivery before 37 completed weeks of gestation and has a global prevalence of 9.6 %. It is the leading cause of neonatal morbidity and mortality and is responsible for approximately 70 % of all neonatal deaths and 40 % of childhood neurological morbidities **(Fernando et al., 2015).** It had been reported that, presence of higher amounts of IL-8 in different biological body fluids (e.g., maternal and/or fetal blood, amniotic fluid, urine, cervical and/or vaginal secretions and placental tissue) is an independent risk factor for preterm labor **(Hashemi and Shahshahan, 2014).** N-acetylcysteine (NAC) is an acetylated cysteine residue, and gaining primary importance of its action to protect cell against oxidative stress **(Kerksick and Willoughby, 2005).** Oral NAC administration to females with previous preterm birth and along with progesterone after 16 weeks of pregnancy was found to protect against preterm birth recurrence and improve neonatal outcome **(Shahin et al., 2009).**

There is a relationship between increased risk of spontaneous preterm delivery and high IL-8 levels **(Tosun et al., 2010; Rode et al., 2012; Cemgil Arikan et al., 2012).** However, there is no conclusive data on the early pregnancy cytokine levels and birth outcome **(Elksne et al., 2013).**

This cohort prospective study was conducted in Obstetrics and Gynecology department, Al-Azhar University hospital (New Damietta) at the period from January to November 2015. It included 50 cases, with previous history of idiopathic preterm labor in the gestational age between 24 weeks and 36 weeks. Effect of N-acetylcystiene in prevention of preterm labor by affection on interleukin-8 as pro-inflammatory cytokine was evaluated.

In the current study, the mean age was 26.340 ± 4.119 years. This is in accordance of a study of **Parker et al. (2014),** who found that, the mean age of

mothers who had spontaneous preterm labor is 28.0 ± 6.6 years.

In the current study, studied patients had increased body weight 80.280±13.577 kg and body mass index 30.074±4.611 kg/m^2. Among studies examining associations of pre-pregnancy obesity and spontaneous preterm birth, three reported higher risk **(Wise et al., 2010; Cnattingius et al., 2013; Nohr et al., 2007);** another one reported lower risk **(Hendler et al., 2005),** whereas one found no association **(Parker et al., 2014).** Lack of consistency in findings could be due to lack of adjustment by factors associated with preterm birth such as gestational weight gain **(Cnattingius et al., 2013)** smoking, or illicit drug use **(Hendler et al., 2005),** or due to inconsistent cut points of maternal BMI to define obesity and gestational age **(Parker et al., 2014).**

In the current study, obstetric history revealed that, gravidity was 3.920 ±1.175 and parity was 2.88 ±1.17. **Chen et al. (2014)** reported that, gravidity was 1.74±1.15 and parity was 1.17±0.42. these results are in contradiction to that of the present study and it may be due difference in inclusion and exclusion criteria; as **Chen et al (2014)** included primipara in their study and history of preterm labor wasn't of their inclusion criteria.

We found no major maternal or fetal adverse effects of NAC use, apart from 10% of patients discontinued taking NAC owing to nausea, vomiting, headache and low blood pressure, this agrees with **Shahin et al. (2009),** as they found 11.4% discontinuation rate due to nausea and vomiting.

In the current study it was found that in women who didn't continue on NAC 60% of their neonates were admitted to incubator, while women who continued on NAC 4.4% of their neonates were admitted to incubator, with high significant difference (P-value <0.001). This agrees with the finding of **Shahin et al. (2009),** who in a randomized, double blind, placebo-controlled trial with 280 women between 16 and 18 weeks of pregnancy, found that, females with history of previous preterm and were treated with N-Acetylcysteine 12.5% of their neonates were admitted to NICU, while those who didn't receive N-Acetylcysteine 43.9% of their neonates were admitted to NICU. The mechanisms may have interfered with the inflammatory cascade associated with term and preterm labor **(Young, 2002).**

In the current study, administration of N-Acetylcysteine was found to significantly reduced IL-8 level from 0.887±0.657 to 0.393±0.535 after 12 weeks of NAC treatment (P-value <0.001). This agrees with the finding of **Sahib (2013),** as they found that administration of N-Acetylcysteine caused decreased IL-8 by 72% respectively after using for eight weeks. N-Acetylcysteine, has been suggested to have anti-inflammatory properties by suppressing the activation of nuclear factor kappa (NF-kB) **(Jansson et al., 2005).** In addition, it was found that N-Acetylcysteine inhibited the production of TNF-α, IL-6 and IL-8 in human in vitro **(Gosset et al., 1999).**

In the current study we found that those continued administration of NAC has ability to prolong gestational weeks and reduce risk of preterm labor in pregnant women with history of previous preterm labor. This agrees with the finding of **Amin et al. (2008),** who investigated the use of NAC for the treatment of unexplained recurrent pregnancy loss in a large cohort of patients, as they found that NAC therapy was associated with significant prolongation of gestation in patients with a history of unexplained recurrent pregnancy loss. They explained that as; 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., 2003).** It is also likely that lower expression of angiogenesis related and apoptosis-related genes is associated with recurrent pregnancy loss **(Choi et al., 2003).**

From the previous mentioned data, it was found that oral administration of NAC, which is cheap and well tolerated, for women with previous history of idiopathic preterm birth in the 24th week of pregnancy was able to protect against preterm birth recurrence and improve neonatal outcome through its ability to decreases maternal serum value of IL-8.

References

1. Amin, A.F., Shaaban, O.M., Bediawy, M.A. (2008): N-acetyl cysteine for treatment of recurrent unexplained pregnancy loss. Reprod Bio Med Online, 17: 722-726.
2. Attaurrahman, A.-u.-R., Harvey, K., and Siddiqui, R.A. (2010): Interleukin-8 An Autocrine Inflammatory Mediator. ChemInform, (Wiley-Blackwell), 30: 25.
3. Blencowe, H., Cousens, S., Oestergaard, M.Z., Chou, D., Moller, A.B., Narwal, R., andLawn, J.E.(2012): National, regional, and worldwide estimates of prete m birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. The Lancet, 379: 2162-2172.
4. Buhimschi, I.A., Buhimschi, C.S., Weiner, C.P. (2003): Protective effect of N-acetylcysteine against

fetal death and preterm labor induced by maternal inflammation. Am J Obstet Gynecol, 188: 203-208.
5. Cemgil Arikan, D., Aral, M., Coskun, A., Ozer, A. (2012): Plasma IL-4, IL-8, IL-12, interferon-γ and CRP levels in pregnant women with preeclampsia, and their relation with severity of disease and fetal birth weight. J Maternal-Fetal Neonat Med, 25: 1569-1573.
6. Chen, Y.P., Lu, Y.P., Li, J., Liu, Z.W., Chen, W.J., Liang, X.J., and Hocher, B. (2014): Fetal and maternal angiotensin (1-7) are associated with preterm birth. J Hyperten, 32: 1833-1841.
7. Choi, H.K., Choi, B.C., Lee, S.H., Kim, J.W., Cha, K.Y., and Baek, K.H. (2003): Expression of angiogenesis- and apoptosis related genes in chorionic villi derived from recurrent pregnancy loss patients. Mol Reprod Dev, 66: 24-31.
8. Cnattingius, S., Villamor, E., Johansson, S., Bonamy, A.K.E., Persson, M., Wikström, A.K., Granath, F. (2013): Maternal Obesity and Risk of Preterm Delivery. JAMA, 309: 2362.
9. Elksne, K., Jurka, A., Rezeberga, D. (2013): TNF-α AndIL-8 As Prognostic Markers of Birth Outcome in Overweight and Non-Overweight Pregnant Women. Acta Chirurgica Latviensis, 13: 45-50.
10. Fernando, F., Keijser, R., Henneman, P., van der Kevie-Kersemaekers, A.M.F., Mannens, M.M.A.M., van der Post, J.A.M., Ris-Stalpers, C. (2015): The idiopathic preterm delivery methylation profile in umbilical cord blood DNA. BMC Genomics, 16: 736.
11. Gosset, P., Wallaert, B., Tonnel, A.B., Fourneau, C. (1999): Thiol regulation of the production of TNF-α, IL-6 and IL-8 by human alveolar macrophages. Eur Respir J, 14: 98.
12. Hashemi, L., Shahshahan, Z. (2014): Maternal serum cytokines in the prediction of preterm labor and response to tocolytic therapy in preterm labor women. Adv Biomed Res, 3: 126.
13. Hendler, I., Goldenberg, R.L., Mercer, B.M., Iams, J.D., Meis, P.J., Moawad, A.H., Sorokin, Y. (2005): The Preterm Prediction study: Association between maternal body mass index and spontaneous and indicated preterm birth. Am J Obstet Gynecol, 192: 882-886.
14. Jansson, A.H., Eriksson, C., Wang, X. (2005): Effects of budesonide and N-acetylcysteine on acute lung hyperinflation, inflammation and injury in rats. Vas Pharmacol, 43: 101-111.
15. Jauniaux, E., Hempstock, J., Greenwold, N., Burton, G.J. (2003): Trophoblastic Oxidative Stress in Relation to Temporal and Regional Differences in Maternal Placental Blood Flow in Normal and Abnormal Early Pregnancies. Am J Pathol, 162: 115-125.
16. Jenkins, C., Wilson, R., Roberts, J., Miller, H., McKillop, J.H., Walker, J.J. (2000): Antioxidants: Their Role inPregnancy and Miscarriage. Antioxid Redox Signal, 2: 623-628.
17. Kerksick, C., Willoughby, D. (2005): The Antioxidant Role of Glutathione and N-Acetyl-Cysteine Supplements and Exercise-Induced Oxidative Stress. J Int Soc Sports Nutr, 22:38.
18. Ng, P.Y., Ireland, D.J., Keelan, J.A. (2015): Drugs to Block Cytokine Signaling for the Prevention and Treatment of Inflammation-Induced Preterm Birth. Front. Immunol, 6:166.
19. Nohr, E.A., Bech, B.H., Vaeth, M., Rasmussen, K.M., Henriksen, T.B. Olsen, J. (2007): Obesity, gestational weight gain and preterm birth: a study within the Danish National Birth Cohort. Paediatr Perinat Epidemiol, 21:5-14.
20. Parker, M.G., Ouyang, F., Pearson, C., Gillman, M.W., Belfort, M.B., Hong, X., Wang, X. (2014): Prepregnancy body mass index and risk of preterm birth: association heterogeneity by preterm subgroups. BMC Pregnancy Childbirth, 14: 153.
21. Rode, L., Klein, K., Larsen, H., Holmskov, A., Andreasen, K. R., Uldbjerg, N., Tabor, A. (2012): Cytokines and the Risk of Preterm Delivery in Twin Pregnancies. Obstetr Gynecol, 120: 60-68.
22. Sahib, A.S. (2013): Effects ofOral Antioxidants on Lesion Counts Associated with Oxidative Stress and Inflammation in Patients with Papulopustular Acne. J Clin Exp Dermatol Res, 03:163.
23. Shahin, A.Y., Hassanin, I.M.A., Ismail, A.M., Kruessel, J.S., Hirchenhain, J. (2009): Effect of oral N-acetyl cysteine on recurrent preterm labor following treatment for bacterial vaginosis. Int J Gynecol Obstetr, 104: 44-48.
24. Tosun, M., Celik, H., Avci, B., Yavuz, E., Alper, T., Malatyalioglu, E. (2010): Maternal and umbilical serum levels of interleukin-6, interleukin-8, and tumor necrosis factor-α in normal pregnancies and in pregnancies complicated by preeclampsia. J Maternal-Fetal & Neon Med, 23: 880-886.
25. World Health Organization.(2014): model lists of essential medicines.
26. Wise, L.A., Palmer, J.R., Heffner, L.J., Rosenberg, L. (2010): Prepregnancy Body Size, Gestational Weight Gain, and Risk of Preterm Birth in African-American Women. Epidemiol, 21: 243-252.
27. Young, A. (2002): Immunolocalization of Proinflammatory Cytokines in Myometrium, Cervix, and Fetal Membranes During Human Parturition at Term. Biol Reprod, 66: 445-449.