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ORIGINAL ARTICLE

Maternal plasma levels of interleukin-6, C-reactive protein, vitamins C, E and A, 8-isoprostane and oxidative status in women with preterm premature rupture of membranes

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Abstract

Objective: Preterm premature rupture of membranes (PPROM) is associated with significant maternal and perinatal morbidity. This study examined maternal oxidative stress in PPROM.
Methods: This was a prospective cross-sectional study conducted in a university hospital. A total of 72 pregnant women were recruited into two groups, those with PPROM (38 cases) and those without PPROM (34 controls) matched for gestational age. Plasma interleukin-6, C-reactive protein, vitamins C, E and A, 8-isoprostane, total oxidant status (TOS) and antioxidant status (TAS) were determined for all study participants and the data were compared between the PPROM and control groups.
Results: Both case and control groups were comparably matched in age, parity, gestational age and smoking status. There was a significant association between low 8-isoprostane, low vitamin C and high total oxidant status and the occurrence of PPROM ($p < 0.001$).
Conclusions: Plasma vitamin C and 8-isoprostane levels were lower and TOS higher in women with PPROM. Further research is needed to identify robust biological markers for the prevention and also prognosis of PPROM.

Keywords

8-isoprostane, early membrane rupture, oxidative stress, pregnancy, preterm premature rupture of membranes, total antioxidant status, total oxidant status, vitamin C

History

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Introduction

Preterm premature rupture of the membranes (PPROM) prior to 37 weeks of gestation occurs in approximately 35% of all pregnancies delivering prematurely [1]. The management of a patient who presents with PPROM has still been controversial and remains a challenging event in maternal fetal medicine. Further, PPROM is a crucial complication in obstetric and a major contributor to perinatal morbidity and mortality worldwide, mainly from preterm births [2].

The precise underlying cause of PPROM is not well understood; however, it appears to be multifactorial, including infection, smoking, multiple gestation, cervical incompetence, low socioeconomic status and polyhydroamnios [3,4]. The other underlying factors associated with PPROM are vitamin deficiency, homocysteine and infection-mediated proinflammatory cytokines [5,6]. A growing body of evidence indicated that PPROM may result from reactive oxygen species (ROS)-induced damage to amnion epithelium or collagen synthesis in the chorioamniotic membrane [7–9]. Vitamin C, ascorbic acid, is an effective water-soluble antioxidant that plays a significant role in collagen formation [8]. Vitamin C acts synergistically to scavenge several ROS by reducing oxidative stress [10]. Clinically, low ascorbic acid concentrations have been reported to lead to an increased risk of PPROM [11].

Elevated maternal plasma cytokines in the second trimester and at delivery have been related with adverse pregnancy outcome [12,13]. The chorioamnion is a biologically active membrane, which is vulnerable to reactive oxygen species [14]. Pregnancy is a condition in which the intensified generation of free radicals occurs [15]. *In-vitro* exposure of cultured amniocytes to ROS provides additional evidence of alterations in intracellular biology that could predispose to PROM [16]. Isoprostanes, chemically stable prostaglandin isomers, are mostly produced in the process of non-enzymatic peroxidation of arachidonic acid and of lipoproteins generated by reactive oxygen species [17]. According to several reports, the concentrations of cellular membrane isoprostane may serve as a ubiquitous, sensitive and specific marker of *in vivo* oxidative stress intensity [18,19].

Taken together, whether oxidative stress and antioxidants play a significant role, pregnancy complicated with PPROM remains to be unclear. The present study aims to evaluate total oxidant status (TOS), total antioxidant status (TAS), 8-isoprostane, IL-6 and vitamins A, C and E in women whose pregnancies were complicated by PPROM and normal healthy pregnancies.

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Table 1. Demographic characteristics of patients in PPROM and control groups.

Demographics	PPROM group ($n = 38$)	Control group ($n = 34$)	p
Age, years	28.5 ± 5.9	27.0 ± 4.0	0.22
Gravida, median (IQR)	3 (2–4)	2 (1–3)	0.024
Parity, median (IQR)	1 (0–2)	1 (0–2)	0.09
Previous vitamin supplementation, *n* (%)	19 (51.4%)	17 (51.5%)	0.98
Smoker, *n* (%)	5 (13.2%)	4 (9.1%)	0.44
Gestational age at PROM and blood sample collection, weeks	29.2 ± 3.2	30.0 ± 3.5	0.48
Gestational age at delivery, weeks	31.4 ± 3.2	38.5 ± 1.3	0.0013
Birth weight, g	1700.7 ± 779.6	3015.8 ± 462.5	0.0012
Apgar score at 1st minute, median (IQR)	7.0 (7.0–8.0)	8.0 (8.0–9.0)	0.0013
Apgar score at 5th minute, median (IQR)	10 (8–10)	10 (9–10)	0.07
C-section, *n* (%)	23 (60.5%)	20 (58.8%)	0.97

Values are given in mean ± standard deviation as otherwise stated.
Statistical significance is given as $p < 0.05$.

Methods

Thirty-eight women with singleton pregnancies who were diagnosed with PPROM between 24 and 34 weeks of gestation in the Obstetrics and Gynecology Department in Our University, Medical School Hospital were recruited as the study group. From an unselected population of pregnant women undergoing their routine pregnancy follow-up, 34 gestational age-matched pregnant women who delivered at term were selected to the study as the control group. The study protocol was approved by the Institutional Ethics Committee for Research on Human Subjects. Informed written consent form was obtained from all the participants.

Participant selection criteria

The inclusion criteria for women with a normal healthy pregnancy were (1) no clinical sign of infection; (2) absence of clinical evidence of any major disease, such as maternal cardiac disease, diabetes mellitus, connective tissue disorders and renal or liver failure; (3) singleton pregnancy. The inclusion criteria for the pregnant women with PPROM were: (1) no sign of infection; (2) no fetal abnormalities; (3) singleton pregnancy. All the patients received antibiotics, steroid and were hospitalized for fetal heart monitoring and uterine activity assessment performed daily. The delivery ensued when endometritis signs developed or fetal distress occur, otherwise hospitalization continued up to 34 weeks.

The exclusion criteria from the study were the presence of (1) multiple pregnancies; (2) macrovascular and/or microvascular complications; (3) fetuses with chromosomal, genetic or structural defects; (4) chronic medical diseases, such as urolithiasis, liver cirrhosis, congestive heart failure, hypertensive disorders or other known major diseases.

Biochemical analysis

Venous blood was obtained from arm of each woman in the study group and healthy pregnant controls. The blood samples were collected in potassium EDTA tubes and delivered to the laboratory within 20 min, then centrifuged at 2000*g* for 10 min at 4 °C and the plasma was stored at −80 °C until assayed. Whole white blood cell count and C-reactive protein (CRP) were analyzed as routinely with the Coulter counter (Coulter Microdiff 18, Beckman Coulter Inc., Miami, FL).

Plasma vitamins A, C and E were analyzed with Schimadzu 10A VP HPLC equipment using commercial kits, Immundiagnostik AG, Bensheim, Germany, minimum detectable concentrations being 0.005 mg/L, 0.25 mg/L and 1 mg/L, respectively. The intra- and inter-assay coefficients of variance (CV) for vitamins A, C and E were 2.9 and 4.2%, 1.6 and 2.7%, 3.2 and 4.8%, respectively.

Total antioxidant status, TOS, interleukin 6 (IL-6) and 8-isoprostane analysis was done by Enzyme-Linked Immunosorbent Assay (ELISA) using the commercially available kits, Immundiagnostik AG, Bensheim, Germany, Ani Biotech Oy Orgenium Laboratories Business Unit, Vantaa, Finland and, Cayman Chemical Company, Ann Arbor, MI, minimum detectable concentrations being 130 μmol/L, 7 μmol/L, 7 pg/mL and 4 pg/mL, respectively. The intra- and inter-assay coefficients of variance (CV) for TAS, TOS and IL-6 were 3.9 and 2.6%, 2.9 and 6.6%, 9.4 and 8.6%, respectively. The results were presented as mean ± standard deviation.

Statistical analysis

Comparison between the groups was performed using Mann–Whitney U-test and Student's *t*-test for continuous variables. The normality of distributions was assessed using the Kolmogorov–Smirnov test. Variables (white blood cell count, CRP, vitamin A, vitamin C, IL-6 and 8-isoprostane) with a skewed distribution were log-transformed. For all comparisons, probability of < 0.05 was considered to be significant. The data were analyzed using the Statistical Package for Social Sciences software 19.0 for Windows (SPSS, Inc., Chicago, IL).

Results

Demographic characteristics of patients in each group are presented in Table 1. No differences were noted between the groups regarding age, parity and gestational age at PROM when blood samples were collected. Rate of C-section was 60.5% ($n = 23$) in the PPROM group and 58.8% ($n = 20$) in the control group ($p = 0.97$). Smoking habits were statistically similar between the groups (13 and 9% in the study and control groups, respectively; $p = 0.44$). The rate of previous vitamin supplementation was also similar between the groups (51 and 52% in the study and control

Table 2. The comparison of variables between the groups.

Biochemical markers	PPROM group ($n = 38$)	Control group ($n = 34$)	p
White blood cell count, $\times 10^3$/mm	12.9 ± 0.3	9.8 ± 0.2	0.0014
CRP, mg/L	7.1 ± 9.8	6.9 ± 4.9	0.92
TAS, μmol/L	343.2 ± 25.8	351.2 ± 31.9	0.24
TOS, μmol/L	552.4 ± 217.4	379.6 ± 142.1	0.001
IL-6, pg/mL	97.5 ± 202.6	186.7 ± 320.0	0.21
8-isoprostane, pg/mL	55.6 ± 146.2	130.2 ± 161.6	0.0011
Vitamin C, mg/L	7.29 ± 2.19	13.85 ± 3.07	0.0012
Vitamin E, mg/L	20.38 ± 6.47	18.15 ± 6.38	0.15
Vitamin A, mg/L	0.70 ± 0.57	0.76 ± 0.44	0.65

Values are given in mean ± standard deviation as otherwise stated.
Statistical significance is given as $p < 0.05$.

groups, respectively; $p = 0.98$). Mean gestational age at delivery (31 versus 39 weeks; $p < 0.001$) and neonatal birthweight (1700 versus 3015 g; $p < 0.001$) were significantly lower in the PPROM group compared to the control group, as expected.

The concentrations of maternal blood biochemical markers in both groups are demonstrated in Table 2. Mean levels of maternal serum TOS and 8-isoprostane in the PPROM group were significantly different from those of the control group ($p < 0.001$). No statistically significant difference was noted between the groups regarding CRP, TAS and IL-6 ($p = 0.92$, $p = 0.24$ and $p = 0.21$, respectively).

Vitamin A and E levels were found to be statistically similar in both groups (0.70 ± 0.57 versus 0.76 ± 0.44 and 20.38 ± 6.47 versus 18.15 ± 6.38 in the PPROM and control groups, respectively; $p = 0.65$ and $p = 0.15$, respectively), whereas vitamin C level was observed to be significantly lower in the PPROM group compared to the control group (7.29 ± 2.19 versus 13.85 ± 3.07; $p < 0.001$).

Discussion

The associations between PPROM and both maternal and fetal infections have been well established, and also cross-sectional studies have demonstrated correlations between intra-amniotic infection with elevated intra-amniotic cytokines and defective ROS [20,21]. In the present study, no significant differences were found in maternal plasma IL-6 and TAS levels between the PPROM and control groups, but the maternal plasma 8-isoprostane concentration was found to be significantly lower and the maternal plasma TOS level was higher in pregnant women with PPROM.

At term pregnancy, the rupture of chorioamnion membrane is suggested to initiate in a weakened paracervical region due to collagen remodeling and apoptosis [22,23]. Collagen damage caused by elevated ROS formation and/or antioxidant depletion may affect PPROM process. Associations of PPROM with elevated concentrations of oxidative stress marker in tissue samples of women with PPROM have been demonstrated [8,9]. The present study indicated that pregnant women with PPROM had lower plasma 8-isoprostane than pregnant women who delivered at term. However, the previous study demonstrated that women with complicated pregnancy such as preterm birth had higher urinary level of 8-isoprostane than women with healthy pregnancies, but the plasma level of 8-isoprotane was not different between the groups [24]. In another study, plasma-free isoprostane concentrations in patients with PPROM were found to be significantly higher than those in the preterm control group [25]. However, plasma-free isoprostane concentrations in the preterm control group were also found to be significantly lower than those in patients with PROM at term and control at term group. The explanation for the discrepancy with our results may be that we assessed serum levels of 8-isoprostane, which could be eliminated quickly from the circulation. Further, urinary concentration of oxidative stress markers may reflect better the severity of maternal oxidative stress. Further studies are needed regarding the relation of PPROM with isoprostanes.

In agreement with the previous reports, we found low plasma vitamin C levels in combination with high plasma TOS levels in women with PPROM. Several studies suggested the effect of antioxidants on protecting choriamniotic membrane from damage by ROS [7,9]. *In-vitro* exposure of cultured amniocytes to ROS has provided additional evidence that ROS alter the biology of chorioamniotic membrane [7–9]. Fetal membranes exposed to cigarette smoke extracts showed evidence of oxidative stress and fetal membrane apoptosis [26]. Menon et al. [26] supported the hypothesis that an alternate non-infectious pathway mediated by oxidative stress and apoptosis in preterm prelabor rupture of membranes may promote proteolysis resulting in membrane weakening and rupture. Researchers also demonstrated that deficiency of ascorbic acid contributed to poor collagen production in fetal membranes and has also been reported to lead to an increased risk for PPROM [27]. A recent study found that supplementation of antioxidant vitamin during pregnancy decreased markers of oxidative stress at delivery [28].

In summary, the present data further support the hypothesis that PPROM is related to ROS-induced damage to amniotic membrane. The oxidative stress in pregnancy may augment the risk of PPROM that is coordinated by excessive peroxidation of collagen in chorioamniotic membrane. It may suggest that ROS develops early in pregnancy, thus PPROM may occur depending on the severity and duration of exposure. Further prospective clinical trials may clarify whether oxidative stress markers would be beneficial in the prediction of PPROM in the first trimester of pregnancy.

Declaration of interest

The authors report no conflicts of interests.

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