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Oxidant and antioxidant status in mothers and their newborns according to birthweight

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ABSTRACT

Objective: The aim of this study is to determine the oxidant and antioxidant status in Algerian mothers and their newborns according to birth weight.
Study design: Subjects for the study were consecutively recruited from Tlemcen hospital. 139 pregnant women and their newborns were included. The plasma total antioxidant activity (ORAC), vitamins A, C, E, hydroperoxides, carbonyl proteins, and erythrocyte antioxidant enzyme activities (catalase, glutathione peroxidase, glutathione reductase and superoxide dismutase) were measured on mothers and their newborns. Lipid and lipoprotein parameters were also determined. The results were assessed in accordance with small for gestational age (SGA), appropriate (AGA) and large (LGA) birth weight of the newborn.
Results: SGA newborns and their mothers had low ORAC, vitamin C and E values ($P < 0.01$) and high plasma hydroperoxide and carbonyl protein levels ($P < 0.01$) compared to AGA groups. The SGA group showed also altered erythrocyte antioxidant enzyme activities and several lipid and lipoprotein changes. In LGA compared to control newborns, hydroperoxide, carbonyl protein levels and SOD activity were enhanced while ORAC, vitamin A and E levels were reduced. However, oxidant and antioxidant status in their mothers was similar to that in control mothers.
Conclusion: Oxidative stress is present in both SGA and LGA newborns, with a concomitant alteration in maternal oxidant and antioxidant status only in intrauterine growth restriction.
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1. Introduction

Pregnancy, mostly because of the increased oxygen requirement and mitochondria-rich placenta, is a condition exhibiting increased susceptibility to oxidative stress. Evidence for this concept includes studies demonstrating elevated levels of oxidative stress markers in normal pregnancy [1]. Plasma and erythrocyte malondialdehyde (MDA) levels were significantly higher while erythrocyte glutathione (GSH) levels and superoxide dismutase (SOD) activity were significantly lower in pregnant women in the third trimester than in nonpregnant women [2,3]. The perinatal period and the delivery in particular is a critical time for maintaining a balance between the production of free radicals and the incompletely developed antioxidative protection of the fetus and newborn. Delivery represents a significant oxidative stress for a fetus, which passes from a hypoxic intrauterine space to a normooxic environment [4]. Lipid peroxidation and antioxidant status are changed during delivery, and these changes affect the fetus by creating oxidative stress [2,5]. However, in uncomplicated term pregnancy, appropriate for gestational age (AGA) newborns possess antioxidant defense capable of resisting the physiological oxidative stress at birth [4]. Several studies have addressed the influence of labor and mode of delivery on oxidative stress [5–7]. Oxidative stress may be related to delivery or to a pre-existing fetal oxidative status [7]. Indeed, high oxidative stress was found in the fetal circulation, regardless to the mode of delivery [6].

Oxygen free radicals have been implicated in the etiology of premature delivery, fetal growth restriction, eclampsia, maternal infections and maternal malnutrition [4,8–10]. Risk may, however, depend on the mother's antioxidant status which potentially protects the maternal–fetal unit, thus increasing intrauterine growth and infant weight at birth [2,4,11]. In fact, the steady-state

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formation of ROS produced during cell metabolism is normally balanced by a similar rate of consumption by antioxidants. Under normal conditions, protective intracellular enzymes mainly catalase and superoxide dismutase, and non-enzymatic antioxidants such as glutathione and vitamins A, C and E prevent ROS accumulation. Oxidative stress may result from imbalance in this pro-oxidant–antioxidant equilibrium.

A significant correlation was found between some maternal and cord blood oxidative stress markers [12]. However, few data provided a global estimation of oxidative stress in mothers and their newborns, according to birth weight. During pregnancy complicated by intrauterine growth restriction, the MDA concentration in amniotic fluid was higher and total antioxidant capacity of the serum was lower than in normal pregnancy [13,14]. Oxidative stress was induced both in small for gestational age (SGA) newborns and their mothers which is manifested as increased lipid peroxidation and protein oxidant damage [15]. The implication of oxidative status in large for gestational age (LGA) newborns of healthy mothers is not well known.

Epidemiological studies suggest that there is a relationship between SGA and LGA newborns and developing metabolic syndrome in adulthood [16]. Oxidative stress has been implicated in adult metabolic syndrome [16]. The question of the possible link between fetal oxidant and antioxidant status and the development of long term metabolic abnormalities is of a great interest.

The aim of the present study is to investigate oxidative stress status in AGA, SGA and LGA newborns and in their respective mothers. This status was evaluated by assaying both plasma total antioxidant capacity (ORAC), markers of lipid and protein oxidation and blood antioxidant defenses, namely erythrocyte superoxide dismutase, catalase, glutathione peroxidase and reductase activities, plasma vitamin A, C and E levels.

2. Materials and methods

2.1. Patients

The study population included 139 women giving birth at the obstetrics and genecology department of Tlemcen Hospital, Tlemcen, Algeria. They were recruited successively among the admitted women at the hospital. A written consent was required from all the subjects, and the study was approved by the Tlemcen Hospital Committee for Research on Human Subjects.

The women claimed to have no history of chronic diseases, eclampsia, infections or fetal anomalies. All were tested for gestational diabetes according to the World Health Organization criteria, and all had normal glucose tolerance test during the third trimester and within 48 h of birth. Care was taken to ensure that all the subjects were of similar age, weight, height, gestational age and parity. All these women had uncomplicated singleton pregnancies. None showed any abnormalities during labor and delivered vaginally at term. Gestational age was estimated by the last menstrual period and confirmed by a first-trimester ultrasound scan. Newborn weight was recorded immediately after delivery. Appropriate growth was defined by the presence of ultrasonographic signs (when biparietal diameter and abdominal circumference were between the 10th and 90th percentiles) according to the normograms of Campbell and Thoms [17] and by postnatal confirmation of a birth weight between the 10th and 90th percentiles (between 2600 and 3900 g) according to our population standard curves (unpublished data). Small for gestational age newborns with birth weight less than 10th percentile or less than 2500 g at term, and large for gestational age newborns with birth weight over 4000 g at term (>90th percentile) were also identified.

Table 1
Maternal and neonatal characteristics

Characteristics	Group 1 (AGA)	Group 2 (SGA)	Group 3 (LGA)
Number	56	45	38
Age (years)	25 ± 1	24 ± 1.2	25 ± 1.6
BMI (kg/m^2)	22.80 ± 2.3	22.14 ± 1.7	23.07 ± 1.5
Parity	2 ± 1	2 ± 1	2 ± 1
Gestational age (weeks)	38.50 ± 0.56	38.11 ± 0.50	39.20 ± 0.80
Birth weight (g)	3290 ± 58	2295 ± 33	4384 ± 70
M/F sex ratio	30/26	20/25	22/16

Values are means ± S.E.M. BMI, body mass index (weight/height^2); M/F, males/females.

Three groups were then selected and studied: Group 1 consisted of 56 mothers and their AGA newborns (control group). Group 2 consisted of 45 mothers and their SGA newborns, and Group 3 consisted of 38 mothers and their LGA neonates. Maternal and neonate characteristics are shown in Table 1.

2.2. Blood samples

Maternal fasting blood samples were obtained within 48 h of birth (median time of collection, 20 h) from the arm veins of the mothers. Maternal blood was not taken during delivery when mothers were in active labor to avoid additional discomfort. Collecting the blood samples at this time point did not affect the results since maternal oxidant and antioxidant markers increased from predelivery to 24 h post partum and then decreased significantly only 48 h post partum [3,18]. Immediately after the infant delivery, and before the placenta delivery, the umbilical cord was doubly clamped and mixed venous and arterial cord blood was obtained.

Blood samples were collected in heparinized tubes, centrifuged and plasma was separated for lipids, vitamins, total antioxidant capacity, hydroperoxides and carbonyl proteins determinations. The remaining erythrocytes were washed three times in isotonic saline, hemolysed by the addition of cold distilled water (1/4), stored in refrigerator at 4 °C for 15 min and the cell debris was removed by centrifugation ($2000\,g \times 15$ min). The hemolysates were appraised for antioxidant enzyme activities.

2.3. Chemical analysis

2.3.1. Lipoprotein determination

Plasma lipoprotein fractions (LDL, $d < 1.063$; HDL, $d < 1.21$ g mL^{-1}) were separated by sequential ultracentrifugation in a Beckman ultracentrifuge (Model L5-65, 65 Ti rotor), using sodium bromide for density adjustment.

Plasma triglyceride and total cholesterol, LDL- and HDL-cholesterol contents were determined by enzymatic methods (kit Boehringer, Mannheim, Germany). Plasma apolipoprotein (apo) A-I and apo B100 levels were determined by immunoelectrophoresis.

2.3.2. Scavenging capacity of plasma

The oxygen radical absorbance capacity of plasma (ORAC) employs the oxidative loss of the intrinsic fluorescence of allophycocyanin (APC) as we have previously described [19]. APC fluorescence decay shows a lag or retardation in the presence of antioxidants, related to the antioxidant capacity of the sample. Trolox was used as a reference antioxidant for calculating the ORAC values, with one ORAC unit defined as the net protection area provided by 1 μM final concentration of trolox.

2.3.3. Determination of plasma levels of vitamins A, C and E

Plasma α-tocopherol (vitamin E) and retinol (vitamin A) were determined by reverse phase HPLC and detected by an UV detector at 292 nm for vitamin E and 325 nm for vitamin A. Vitamin C levels

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