ORIGINAL ARTICLE

Neonatal and maternal concentrations of hydroxil radical and total antioxidant system: protective role of placenta against fetal oxidative stress

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Abstract

BACKGROUND: Reactive oxygen species (ROS) may cause peroxydation of lipids, proteins and deoxyribonucleic acids with subsequent cell damage. The hydroxyl radical ($OH^{\bullet}$) represents a measure of global oxidative stress. Hydroxyl radicals are short-lived; they form an important part of radical chemistry nonetheless. The measure of total antioxidant system (TAS) can give useful information about the extent of defence capable of counteracting the oxidative damage. Pregnancy is an important condition that favors oxidative stress in the fetus. Clinical studies indicate a protective mechanism against O_2 toxicity in the human feto-placental unit.

AIM: This study reports the $OH^{\bullet}$ and TAS concentrations in mother and fetus at birth to evaluate the role of the placenta against fetal oxidative stress.

METHODS: Blood samples were collected at delivery from 45 healthy women at term and from their newborns. The maternal and neonatal $OH^{\bullet}$ and TAS concentrations were compared by paired Student *t*-test.

RESULTS: $OH^{\bullet}$ was higher in maternal blood than in cord blood (573.75± 170.0 UCarr/l vs 40.08±33.37 UCarr/l) ($p<0.01$); TAS concentrations did not differ between the two groups (1.11±0.09 mmol/l vs 1.17±0.12 mmol/l). Multiple regression analyses: maternal and neonatal $OH^{\bullet}$ decreases with maternal age; only maternal TAS and $OH^{\bullet}$ are related to gestational age in a nonlinear fashion. Female infants showed higher values of maternal and neonatal TAS as compared to male infants.

CONCLUSION: TA protective role of the placenta against oxidative damage is in keeping with a large enough gradient of ROS (between mother and fetus) and the passage of TAS from mother to fetus.

Abbreviations:

ROS	- reactive oxygen species	OHP	- organic hydroperoxides
TAS	- total antioxidant system	VD	- vaginal delivery
MDA	- malondialdehyde	CS	- caesarean section

INTRODUCTION

The oxygen-free radicals (or reactive oxygen species, ROS) are highly reactive compounds, particularly the superoxide anion (O_2^-) and the hydroxyl radical OH•. The ROS attack cellular and interstitial substances to produce oxidation of fatty acids, proteins and deoxyribonucleic acids through a series of reactions called "oxidative stress".

All these reactions may produce structural and functional alterations which cause cell death (Halliwell 1989; Sies 1991; 1993). Newborns and particularly preterm infants are at high risk for oxidative stress at birth and are very susceptible to oxidative damage by ROS because the extrauterine environment (pO_2: 100 mmHg) is richer in oxygen than the intrauterine environment (pO_2: 20–25 mmHg). This 4- to 5-fold increase is exacerbated by the low efficiency of the natural antioxidant system in newborns, especially in preterm newborns (Frank & Sosenko 1987; Kelly 1993; Saugstad 1996; Buonocore *et al.* 2000; 2001; 2002). Moreover, the imbalance between antioxidant- and oxidant-generating systems results in oxidative stress at birth, as suggested by reliable markers of increased ROS release in severely ill newborns (Kelly 1993; Rice-Evans & Gopinathan 1995; Pitkanen *et al.* 1993; Varsila *et al.* 1994; Supnet *et al.* 1994; Schmidt *et al.* 1996; Saugstad 1990; Gitto *et al.* 2009).

Previous studies showed that umbilical cord blood concentrations of lipid peroxides such as malondialdehyde (MDA) and organic hydroperoxides (OHP) are increased in acidemic conditions and in situations known to lead to intra-partum hypoxia (Wang *et al.* 1996; Wang & Rogers 1997a;b; Mongelli *et al.* 1997; Rogers *et al.* 1998; Gveric-Ahmetasevic *et al.* 2009). It is difficult to identify the free radicals in biologic systems because of their high instability, so the activity of ROS may be evaluated by measuring their oxidative products such as MDA and OHP (Lunec 1992). These tests, however, lack specificity for measurement of oxygen radicals (Gutteridge & Quinlan 1983).

Moreover, OH•, the neutral form of hydroxide ion, represents the global oxidative stress because it is at the beginning of the oxidation of lipids, proteins and DNA. Hydroxyl radicals are highly reactive and, as a consequence, short-lived; they form an important part of radical chemistry nonetheless (Erel 2004). Human pregnancy is associated with hyperlipidemia and oxidative stress due to increased oxygen demand that augments the rate of production of lipid hydroperoxides and other ROS like as OH• (Gitto *et al.* 2009; Ethier-Chiasson *et al.* 2008; Milczarek *et al.* 2008).

The measurement of both OH• and total antioxidant system (TAS) in mother and newborn at birth could provide useful information about the mechanisms capable of interrupting or limiting fetal oxidative stress. In this study, we compared OH• and TAS concentrations in maternal blood and cord blood at birth in order to investigate the protective role of the placenta against fetal oxidative stress. Moreover, we evaluated the circadian variation of these systems in newborns because in adulthood the antioxidant system shows a circadian periodicity (Luo *et al.* 1997).

Tab. 1. Characteristics of the patients.

	(mean± SD)
Maternal age (y)	30.77 ± 4.78
Gestational age (wks)	38.60 ± 1.48
Gender	24 M/24 F
Birth-weight (g)	3082.92 ± 429.38
1-Min Apgar score	8 ± 1
5-Min Apgar score	9 ± 1

MATERIALS AND METHODS

Patients

Fifty healthy women with uncomplicated pregnancies between 35 and 41 weeks of gestational age, consecutively admitted at the Obstetrician Division, V. Buzzi Children's Hospital of Milan, were considered elegible. Forty-five women (33 vaginal delivery, VD and 12 elective caesarean section, CS in room-air and under epidural anesthesia) were enrolled (Table 1). The indications for elective CS were breech presentation, previous CS or twins. There were three pairs of non-identical twins. The study was approved by the V. Buzzi ethics commitee and informed written consent was obtained from all mothers. All babies were apparently healthy and without signs of asphyxia.

Methods

Immediately after delivery, a maternal venous blood sample was obtained and simultaneously a segment of umbilical cord was double-clamped and heparinized blood samples were drawn from the umbilical artery. All blood samples were immediately centrifuged for 5 min at 4,000 rpm. Then, the plasma and buffy coat were separated. The plasma from each blood sample was collected and stored at –40 °C prior to assay for OH• and TAS concentrations. OH• concentrations were evaluated by d-ROMs Kit (Diacron srl, Italy), a spectrophotometric procedure which estimates the total amount of hydroxyl radicals present in a 10-L blood sample. The results were expressed in conventional arbitrary units, called Carr units. The value of one Carr unit corresponds to a concentration of 0.08 mg/dl of OH•. The normal range in adults is 250–300 UCarr/l. Within-run variation was less than 2.6% and between-run variation less than 4.6%. TAS concentrations were measured by Randox, a spectrophotometric method which estimates the total antioxidant system (i.e. GSH, thiols, proteins, bilirubin, uric acid, cholesterol, β-carotene, ascorbate,

vitamin E, *etc*). The normal range is > 3.5 mmol/l in adults (Carratelli *et al.* 2001).

Statistical analysis

The maternal and neonatal OH• and TAS concentrations, expressed as mean±SD, were compared by paired Student *t*-test. Results were considered to be statistically significant when $p<0.05$. Multiple regression analyses were performed with maternal OH• and TAS and neonatal OH• and TAS as dependent variables, and gender (g), birth weight, gestational age, and maternal age as independent variables. Whenever a given variable was found to depend with statistical significance on any of the factors recorded, residuals were computed from the regression model. Because there may have been gender differences for some variables, rhythmometric analyses were applied separately for boys and girls as well as for all newborns irrespective of gender. Rhythmometric analyses consisted of the least squares fit of cosine curves with periods of 1 year and 1 day, and their corresponding harmonic terms (Bingham *et al.* 1982).

RESULTS

Out of original 50 paired blood samples studied, 5 (10%) were excluded from analysis because of incomplete data. OH• concentrations were significantly higher in mothers than in arterial cord blood ($p<0.01$) while TAS concentrations did not differ between the groups (Table 2). Maternal and neonatal OH• were found to decrease as a function of maternal age (r=–0.355, $p=0.017$ and r=–0.308, $p=0.039$, respectively) (Figures 1a and 1b). Both maternal OH• and TAS were found to vary as a function of gestational age (OH•: $p=0.039$; TAS: $p<0.001$). The relation was found to be nonlinear, and could be approximated by a second-order polynomial. Values were found to be lower at lower gestational age (Figures 2 and 3). There was no correlation between neonatal TAS and birth weight. As compared with vaginal delivery, caesarean section was associated with lower maternal TAS (0.95±0.07 vs. 1.12±0.02; Student t=2.330, $p=0.034$). Female infants showed a tendency toward higher values of maternal and neonatal TAS as compared to males (maternal TAS: 1.11±0.02 vs. 1.02±0.05, Student t=1.692, $p=0.100$; neonatal TAS: 1.20±0.03 vs. 1.13±0.03, Student t=1.855, $p=0.071$).

Rhythmometric analyses were carried out on the original determinations and on residuals, computed to account for associations with maternal age and gestational age described above. Residuals were computed as follows: Maternal OH• was regressed as a function of maternal age ($p=0.007$), gestational age ($p=0.027$) and (gestational age)^2 ($p=0.030$) (overall model: F=5.475; $p=0.003$). Neonatal OH• was regressed as a function of maternal age, and maternal TAS was regressed as a function of gestational age and (gestational age)^2 (overall: F=11.736; $p<0.001$).

Rhythmometric analyses of all 45 determinations indicate the statistical significance of a circasemiannual variation for maternal OH• (PR=14%; $p=0.038$) and for neonatal OH• (PR=13%; $p=0.048$). Neonatal OH• is also characterized by a statistically significant circannual variation (PR=14%; $p=0.039$). When the data are analyzed separately for males and females, the circannual component of maternal OH• is statistically significant for females (PR=45%; $p=0.004$), but not for males.

Tab. 2. OH• and TAS concentrations in maternal and cord blood (mean ± SD).

	Maternal blood	Cord blood	*p*-value
OH• (UCarr/l)	573.75 ± 170.00	40.08 ± 33.37	< 0.01
TAS (mmol/l)	1.11 ± 0.09	1.17 ± 0.12	ns

OH• =mean concentration of hydroxyl radicals
TAS =mean concentration of total antioxidant system
1 Carr unit= concentration of 0.08 mg/dl of hydroxyl radicals.

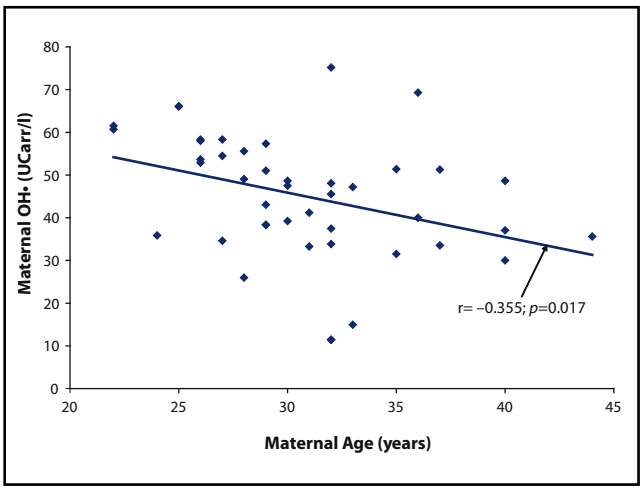

Fig. 1a. maternal OH• concentrations and maternal age.

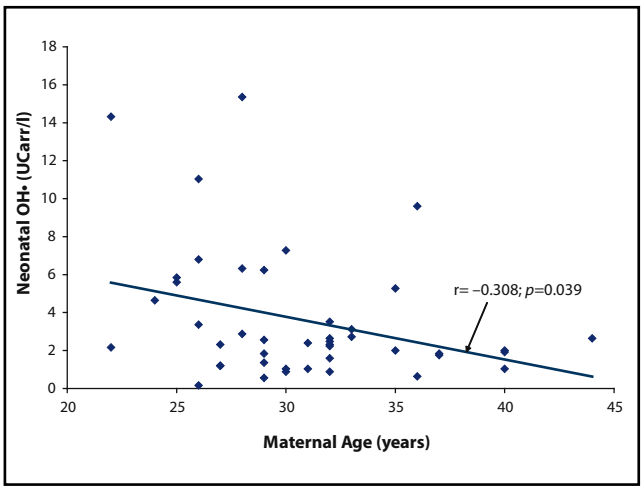

Fig. 1b. neonatal OH• concentrations and maternal age.

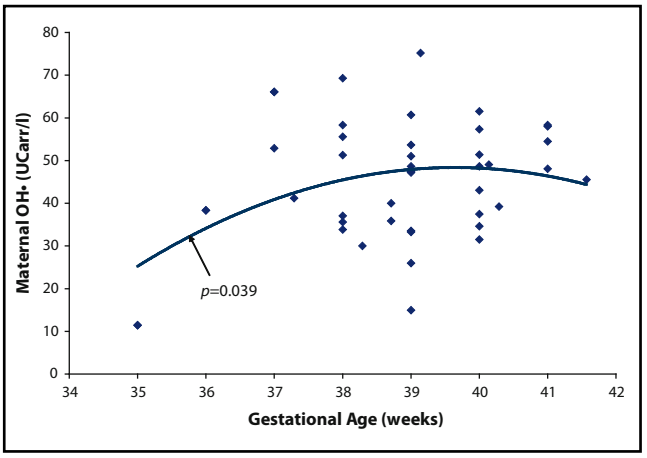

Fig. 2. Maternal OH• concentrations and gestational age.

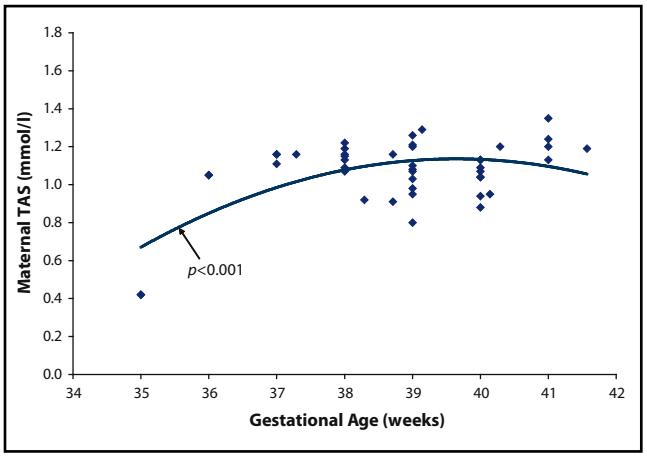

Fig. 3. Maternal TAS concentrations and gestational age.

Analysis of the original determinations shows that both the circannual and circasemiannual components of neonatal OH• are detected with statistical significance for females (1.0-y: PR=32%, p=0.027; 0.5-y: PR=28%, p=0.043), but not for males.

The circadian variation is less well expressed. Analysis of the original determinations indicates that the circadian rhythm is statistically significant only for maternal OH• in females (PR=30%, p=0.034), for neonatal OH• in females (PR=29%, p=0.039), and for maternal TAS in females (PR=35%, p=0.017). It is also of borderline statistical significance for neonatal TAS in males (PR=24%, p=0.063), in which case the circasemidian component is also contributing (PR=25%, p=0.058). Analysis of the residuals also finds a circadian component of borderline statistical significance for maternal OH• in females (PR=27%, p=0.052) and for neonatal OH• in females (PR=21%, p=0.111); the circadian component of maternal TAS in female infants remains statistically significant (PR=32%, p=0.026).

When both the circannual and circadian components are fitted concomitantly to the original determinations, in an attempt to compensate for the uneven distribution of the data along the scales of the year and the day, a model of borderline statistical significance is obtained only for neonatal OH• (1.0 year: PR=13%, p=0.036; 1.0 day: PR=10%, p=0.108; overall: PR=23%, p=0.027). For females, but not for males, maternal TAS can also be described by a combination of the two components (1.0 year: PR=13%, p=0.103; 1.0 day: PR=38%, p=0.011; overall: PR=50%, p=0.014). Similar analyses on the residuals detect with statistical significance the circannual component of neonatal OH• (p=0.033) and the circadian component of TAS of girls (p=0.028).

DISCUSSION

Free radicals are involved in several diseases both in adults (e.g. cardio-vascular diseases, neoplasia, Parkinson and Alzheimer) and in infants (e.g. retinopathy of prematurity, chronic lung disease) (Kelly 1998; Gibson *et al.* 2000; Magsino *et al.* 2000; Aydin *et al.* 2001; Saugstad 1988; 1996; 2001; Davis 2002; Dani *et al.* 2004; Liu *et al.* 2009).

During pregnancy, the concentration of lipoperoxides increases as pregnancy advances; the concentrations are statistically significantly higher in pregnant women in the third trimester than in non-pregnant women, and the highest concentrations are observed during delivery (Gitto *et al.* 2009; Ethier-Chiasson *et al.* 2008; Milczarek *et al.* 2008, Yoshioka *et al.* 1990; Takehara *et al.* 1990; Uotila *et al.* 1991; Walsh & Wang 1993; Arikan *et al.* 2001). Moreover, the concentration of lipoperoxides was higher in maternal blood than in cord blood at birth, and a multiple regression analysis with maternal MDA as dependent variable showed that the only statistically significant determinant was the cord arterial MDA (Rogers *et al.* 1999).

In keeping with these data, we showed that OH• was higher in maternal blood than in cord blood at birth. Multiple regression analyses of our data demonstrated that maternal and neonatal OH• both decrease with maternal age, while only maternal TAS and OH• are related to gestational age in a nonlinear fashion, perhaps in association with placental maturation and aging.

These findings confirm that oxidative stress increases as pregnancy advances and the at-term infant is protected by an increase in maternal TAS.

We knew that preterm infants are at high risk for damage due to oxidative stress because of lower TAS concentrations (Dani *et al.* 2004; Bracci *et al.* 2002); but in our study, we did not analyze preterm infants, a fact that may account for the lack of correlation between gestational age and neonatal TAS and OH• concentrations.

We showed that gender is an important factor: in fact, our data suggest that females are less susceptible to oxidative stress because they show higher values of maternal and neonatal TAS as compared to males.

Rhythmometric analyses showed that maternal OH• presented circannual (only for female babies) and cir-

casemiannual components, and that neonatal OH• presented both circannual and circasemiannual components only in female newborns. These findings do not offer a clinical explanation, but effects of half-yearly and yearly changes in geomagnetics on human longevity, fetal growth and height in adults have been reported (Wohlfahrt *et al.* 1998).

Previous studies have demonstrated that the placenta is a main source of circulating lipid peroxides during pregnancy because the placental secretion of lipid peroxides was statistically significantly greater toward the maternal side of the placenta than toward the fetal side; these data indicate that the placenta secretes lipid peroxides primarily into the maternal circulation and this secretion does not occur in fetal circulation under usual circumstances (Walsh & Wang 1993; Walsh *et al.* 1996).

On the other hand, the antioxidant mechanisms are multifactorial and complex; in fact, some authors reported increases or decreases of antioxidant substances during uncomplicated pregnancy (Yoshioka *et al.* 1990; Takehara *et al.* 1990; Uotila *et al.* 1991; Carone *et al.* 1993). Other authors observed that only GSH concentrations were statistically significantly lower in pregnant women than in non-pregnant women, and the lowest concentrations were observed at the time of delivery, while other antioxidants (such as GSH-P and GSH-R) did not differ between the two groups (Arikan *et al.* 2001; Zachara *et al.* 1993).

In our study, TAS presents similar concentrations in maternal blood and in cord blood at birth. Arikan *et al.* showed a statistically significant positive correlation in erythrocyte (GSH-P) and glutathione reductase (GSH-R) activities between maternal and cord blood erythrocytes and between maternal erythrocyte GSH-P and cord blood glutathione (GSH) concentrations; furthermore, there was a statistically significant negative correlation between maternal erythrocyte MDA and cord erythrocyte GSH-R concentrations.

In conclusion, our preliminary data in keeping with other published work suggest that: 1) the placenta protects the fetus against the damage due to free radicals by maintaining a large enough gradient of ROS between the two circulations or by actively removing them from the fetal circulation; 2) TAS during delivery may be shared between mother and fetus, and the placenta does not limit the passage of TAS from mother to fetus; 3) gender has an effect on oxidation: in fact, female infants are better protected against oxidative stress as compared to males.

Further studies could be directed at identifying the mechanisms underlying the protective role of the placenta toward the fetus in order to evaluate their oxidative status, to choose the optimal timing of delivery and to design the best neonatal assistance strategy.

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