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Oxidative stress in small-for-gestational age (SGA) term newborns and their mothers

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Abstract

This study used malondialdehyde (MDA) determination by HPLC and enzymatic assays for total serum peroxides and antioxidant capacity to evaluate oxidative stress in 47 healthy full-term small-for-gestational age (SGA) newborns vs 67 appropriate-for-gestational age (AGA) newborns. Blood samples were collected at delivery from umbilical cord artery and vein and from peripheral blood of the babies on the third day after birth. Blood samples of mothers were also collected and compared with blood of 29 normal non-pregnant women (NPW). Serum peroxide values were significantly higher in both groups of mothers than in NPW, decreasing towards the third day in AGA mothers, while persisting in SGA mothers. Antioxidant capacity of sera of both groups of mothers was lower than NPW. Both SGA mothers and babies had increased MDA at delivery, unlike AGA counterparts. MDA levels in umbilical vein were higher than in umbilical arteries, while immunohistochemistry revealed abundant presence of 4-hydroxynonenal (HNE)-protein adducts only in stroma of the SGA placenta. These results show that both mothers and babies are exposed to oxidative stress during and after delivery, which is more pronounced and persistent in the perinatal period of the SGA group, while lipid peroxidation in placenta could play a role in SGA pathophysiology.

Keywords: *Oxidative stress, lipid peroxidation, malondialdehyde, 4-hydroxynonenal, peroxide, antioxidants, small-for-gestational age, appropriate-for-gestational age newborns*

Introduction

The reduction–oxidation balance resulting in the excess of reactive oxygen species (ROS) is important for development of numerous pathological processes, in particular if associated with lipid peroxidation [1]. Therefore, oxidative stress might be relevant for various metabolic processes and modulation of signaling pathways affecting human (patho)physiology from intrauterine development throughout the lifetime [2–4]. In favour of this are findings on bioactivities of products of lipid peroxidation, such as 4-hydroxy-2-nonenal (HNE), which can influence cell proliferation and differentiation at low, physiological concentrations, causing apoptosis and necrosis if increased to supraphysiological values. HNE exhibits a wide range of biological activities based on its affinity to bind to biologically important molecules, including

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inhibition of protein and DNA synthesis, inactivation of enzymes, stimulation of phospholipase C, reduction of gap-junction communication, modulation of the expression of various genes, including c-myc and globin genes, procollagen type I, c-myb, c-fos and transforming growth factor β1 gene, while it also affects glutathione metabolism and induces generation of hydrogen peroxide [5–11]. Hence, while HNE is considered as a mediator of oxidative stress and a signalling molecule, its physiological roles are intensively studied further. Although HNE is nowadays considered as a major bioactive marker of lipid peroxidation, it is likely that the other products of lipid peroxidation such as malondialdehyde (MDA) could also have some regulatory functions which have not been studied yet. Together with the increase of knowledge on redox signalling and physiology of ROS the findings on physiology and pathology of lipid peroxidation could help understanding links between intrauterine and perinatal oxidative stress with development of various disorders associated with oxidative stress during the lifespan.

A process of childbirth may be considered as oxidative stress since it is accompanied by shifting reduction–oxidation balance to oxidative processes [12–16]. Some pathological conditions such as pre-eclampsia and hypertension in pregnancy are in particular connected with increased oxidative stress and neonatal low birth weight, while they mostly imply oxidative stress as a pathological process not during the birth but in the intrauterine development [17–20]. Increased oxidative stress was found also in small for gestational age newborns (SGA) born by malnourished mothers, while the association of oxidative stress with SGA was not clarified [21]. On the other hand, it is known that some children and young adults who were born as SGA developed metabolic syndrome and neurological disability [22–24]. The most recent studies connect increased systemic oxidative stress in premature newborns and babies of low birth weight with some diseases of children and adolescent men [25–28], indicating that oxidative stress might play an important role also in SGA itself. However, Hillestrom et al. [29] did not find differences in the values of markers of oxidative stress between young adults of low birth weight and those of normal birth weight, while they found significant correlations between body mass index in low birth weight adults and the DNA oxidative damage in adolescence. These observations indicate an association of oxidative stress for SGA children with adolescent development of disorders which are associated with oxidative stress such as hypertension, diabetes and metabolic syndrome [22,23,27,28].

Hence, while mechanisms underlying these associations are still unknown and mostly focus on intrauterine oxidative stress as a cause of growth alteration, it is reasonable to assume that oxidative stress could be an important link between adverse prenatal as well as perinatal environment and increased morbidity later in life.

Therefore, the aim of our study was to investigate the markers of oxidative stress and lipid peroxidation status at delivery and on the third day of the perinatal period in full-term SGA and appropriate-for-gestational age (AGA) newborns and their mothers in a view of overall influence of oxidative stress on prenatal environment and perinatal newborn status.

Materials and methods

Study population

The study included newborns of gestational age from 37–40 weeks and their mothers (in total $n = 114$) and

Table I. Group attributes.

Attribute	SGA ($n = 47$)	AGA ($n = 67$)	p
Mother's age (y)	30.4 ± 5.56	30.3 ± 5.88	NS ($p = 0.986$)
Multipara	44%	62%	NS ($p = 0.120$)
GA (wk)	38.97 ± 1.11	39.36 ± 0.81	NS ($p = 0.115$)
BW (grams)	2379.78 ± 366.93	3574.26 ± 453.34	$p < 0.0005$
BL (cm)	46.04 ± 2.067	50.97 ± 1.98	$p < 0.0005$
Mother's height (cm)	167.18 ± 6.19	167.37 ± 7.57	NS ($p = 0.636$)
Mother's weight (kg)	76.8 ± 14.59	78.69 ± 10.57	NS ($p = 0.213$)
Apgar 1st min	Median 10; range 3	Median 10; range 3	NS ($p = 0.980$)
Apgar 5th min	Median 10; range 1	Median 10 (range 2)	NS ($p = 0.817$)
First and second stage of labour (h)	3.575 ± 5.87	4.01 ± 4.4	NS
Smoking (yes)	10–21.3%	9–13.4%	NS ($p = 0.223$)
Ponderal index male (BWg/BLcm^3 × 100)	Median2.3; range 0.6; 10thc 2.14; 90thc 2.6	Median2.7; range 0.7 10thc 2.35; 90thc 2.95	$p < 0.0005$
Ponderal index female	Median 2.5; range 0.8 10th c 2.2; 90th c 2.7	Median 2.7; range 0.6 10th c 2.4; 90th c 3.0	$p < 0.0005$
Cesarion section (n)	25	16	$p = 0.001$

*values are given as mean ± SD, except values which are given as median.

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29 non-pregnant healthy women at generative age (Table I). The newborns were classified with regard to the standard growth chart. SGA newborns had birth weight below the 10th percentile according to gender, gestational age and mother's parity.

The study included healthy women with single pregnancies, without complicated pregnancies and chronic diseases, as well their healthy newborns without acute hypoxemia, chromosomal aberrations, malformations or acute illness. The AGA and the SGA mothers did not show any obvious differences in socio-economic status, they were employed and did not have any social problems. None of them had any nutritional problems.

A higher number of SGA newborns were delivered by caesarean section ($p = 0.001$) than in the control group due to the obstetric history (foetus with growth restriction).

The study was approved by the Ethical Committee of the University of Zagreb. Informed consent was obtained from all women.

Peripheral venous blood samples were taken from women immediately after delivery. Samples from umbilical cord artery and vein were drawn from double-clamped segment of umbilical cord before the first breath of the neonate was taken. We took the blood from the peripheral vein of mothers and newborns at the third day after labour. The samples of sera and EDTA plasma were stored at -80°C.

Malondialdehyde determination by HPLC method

MDA standards were prepared using TEP (1,1,3, 3-tetraethoxy-propane, Sigma, USA) by serial dilution: 0, 0.31, 0.63, 1.25, 2.5, 5 and 10 μM. EDTA plasma samples were analysed as previously described [30]. The total volume of sample (25 μl) or standard was mixed with 225 μl of water, 375 μl 0.44 M H_3PO_4 (Kemika, Croatia) and 125 μl 42 mM TBA (Sigma, USA). All samples were boiled at 100°C for 60 min, cooled on ice and treated 1:1 with alkaline methanol (4.5 ml 1 M NaOH + 50 ml methanol); 50 μl of clear supernatant was analysed after centrifugation by an HPLC method with fluorescence detection (527 nm ex, 550 nm em). The HPLC system consisted of Beckman System Gold, Midas Spark Holland autosampler and a Shimadzu RF-535 fluorescence detector. The mobile phase consisted of 50 mM KH_2PO_4 (Kemika, Croatia), pH 6.8, with 40% (v/v) methanol (Merck, Germany). The flow was set to 1 ml/min and the samples were analysed on a Waters Spherisorb ODS2, 5 μm, 4.6 × 150 mm column.

Total serum antioxidant capacity measurement

Enzymatic *ANTIOX-CAP* assay (AOC—Dr Tatzber KEG, Vienna, Austria) was used to test ROS scavenging properties of serum antioxidants using uric acid standards [31]. Briefly, 10 μl of sera were added into each well of the 96-microwell plate in duplicates and mixed with 100 μl of Reagent 2 containing 0.0003% (v/v) hydrogen peroxide. The absorbance was measured at 450 nm (Easy-Reader 400 FW, SLT Lab Instruments GmbH, Austria). Afterwards, 100 μl of Reagent 1 containing 1.25 mU horse radish peroxidase (HRP) followed by TMB (tetramethylbenzidine) was added. After 15 min of incubation and addition of 50 μl of the Stop Reagent, the absorbance was measured at 450 nm. Results were presented as a difference between the second and the first absorbance values in relation to the uric acid standards.

Total serum peroxide measurement

Serum peroxide concentrations (POX—Dr Tatzber KEG, Vienna, Austria) were determined by a rapid *in vitro* diagnostic assay ('Peroxide-activity' assay; LDN, Germany) as previously described [32]. The assay is based on a peroxide/peroxidase reaction using TMB as chromogenic substrate. Peroxide levels are expressed as 'μM H_2O_2 equivalents' based on ranging values of fresh peroxide solutions as standards. Ten microlitres of the serum samples of unknown peroxide content were placed into the 96-well plates in duplicates and incubated at 37°C with a mixture of defined HRP activity, TMB chromogene and phosphate buffer for 30 min and absorbance was determined in a multiwell plate reader (Easy-Reader 400 FW, SLT Lab Instruments GmbH, Austria) at 450 nm.

Immunohistochemistry for the 4-hydroxynonenal (HNE)-protein adducts in placenta

Immunohistochemistry for HNE-modified proteins was carried out on formalin-fixed paraffin embedded placental tissue samples using monoclonal antibodies obtained from culture medium of the clone 'HNE 1g4', produced by a fusion of Sp2-Ag8 myeloma cells with B-cells of a BALB-c mouse immunized with HNE-modified keyhole limpet hemocyanine. The antibody is specific for the HNE-histidine epitope in HNE-protein (peptide) conjugates and gives only 5% cross-reactivity with HNE-lysine and 4% with HNE-cysteine [33,34].

Immunohistochemistry was done in a three-step procedure as described before [35,36] using a LSAB kit (DAKO, Denmark), where the first step was incubation with anti-HNE monoclonal antibodies (dilution 1:10) during 2 h in humid chambers at room temperature. The second step was incubation with biotinylated secondary goat anti-mouse and anti-rabbit immunoglobulins (AB2) during 30 min. The third step was incubation with streptavidin peroxidase during 30 min. Finally, the reaction was visualized by

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a DAB (3,3-diaminobenzidine tetrahydrochloride in organic solvent) giving a brown colour after 10 min, using haematoxylin contrast staining (blue). Negative control was done on one histological slice of the some tissue, without application of HNE-histidine-specific monoclonal antibodies. Intensity and distribution of the HNE-immunostaining was evaluated semi-quantitatively. The absence of immunopositivity was marked with (0), while with (1) we marked weak immunopositivity in less than 25% of the cells, with (2) medium immunopositivity in 25–50% of cells, and finally with (3) strong immunopositivity in more than 50% of cells. All immunohistochemical analyses were done by a pathologist experienced in the HNE-immunohistochemistry without prior knowledge of the study group design.

Statistical analysis

Results were reported as the mean and standard error. Statistical analysis was performed by the two-tailed non-parametric tests; Mann-Whitney U-test, Wilcoxon test, Chi-square test and Spearman correlation test.

Results

The differences between SGA and AGA groups' characteristics are represented in Table I. The mothers were of similar weight and height. There was no acute hypoxia assessed by Apgar score in any group, while birth weight, birth length and ponderal index showed significant differences. The failure in labour progress was a caesarean section indicator. We found significant differences between SGA and AGA groups according to the type of labour termination ($p = 0.001$), but without any influence on markers of oxidative stress.

The findings on biomarkers of oxidative stress and antioxidant status are outlined in Figures 1–3.

As can be seen from Figure 1A, the SGA newborns showed a much higher level of lipid peroxidation than AGA newborns in the umbilical artery as well as in umbilical venous blood ($p = 0.039$ and $p = 0.003$, respectively), while there was no difference between the newborns of the two groups on the 3rd day after birth ($p > 0.05$).

Similarly, mothers of SGA newborns showed an increase of lipid peroxidation in comparison to the mothers of the AGA newborns at delivery and on the third day after labour ($p = 0.007$ and $p = 0.016$). The mothers of SGA newborns also had higher MDA values than non-pregnant women (Figure 1B).

While the levels of MDA in umbilical artery, umbilical vein and in newborns on the 3rd day of life in the SGA group did not show any differences in comparison with their mother's blood at delivery ($p = 0.61$; $p = 0.27$; $p = 0.394$, respectively), MDA values in AGA's umbilical vein were lower than in their mothers at delivery ($p = 0.001$).

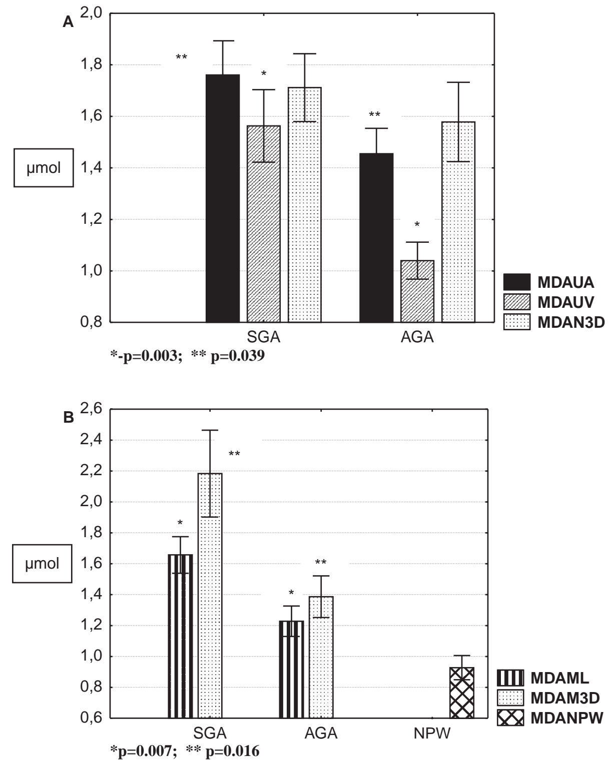

Figure 1. Malondialdehyde in the blood of newborn babies and their mothers. (A) MDA in the blood of newborns. (B) MDA in the peripheral blood of mothers. SGA, small for gestational age; AGA, appropriate for gestational age; NPW, non-pregnant women; MDAUA, MDA in umbilical artery; MDAUV, MDA in umbilical vein; MDAN3D, MDA in newborns 3rd day of life; MDAML, MDA in mother at delivery; MDM3D, MDA in mother 3rd day after labour; MDANPW, MDA in non-pregnant women. Values are given as mean ± SE.

The results of the total serum peroxide determinations are given in Figure 2. As can be seen in Figure 2A, concentrations of peroxides were very low in umbilical cord blood of both groups, without any significance between SGA and AGA. However, both groups had significantly lower levels in umbilical artery and umbilical vein than their mothers at delivery ($p < 0.0005$). Levels were similar for newborns on 3rd day of life ($p = 0.001$).

Opposite to their children who had hardly detectable amounts of peroxides in the blood, the mothers had very increased concentrations of peroxides both at delivery and on the 3rd day after in comparison to the non-pregnant women ($p < 0.0005$). The mothers of SGA newborns had higher peroxides levels than did mothers of AGA newborns both on delivery and on the 3rd day after labour ($p = 0.003$). It should also be noticed that the peroxide levels in the blood of mothers of the AGA newborns decreased on the 3rd day in comparison to delivery, while in the blood of mothers with SGA newborns it persisted to be as high as on the day of delivery (Figure 2B).

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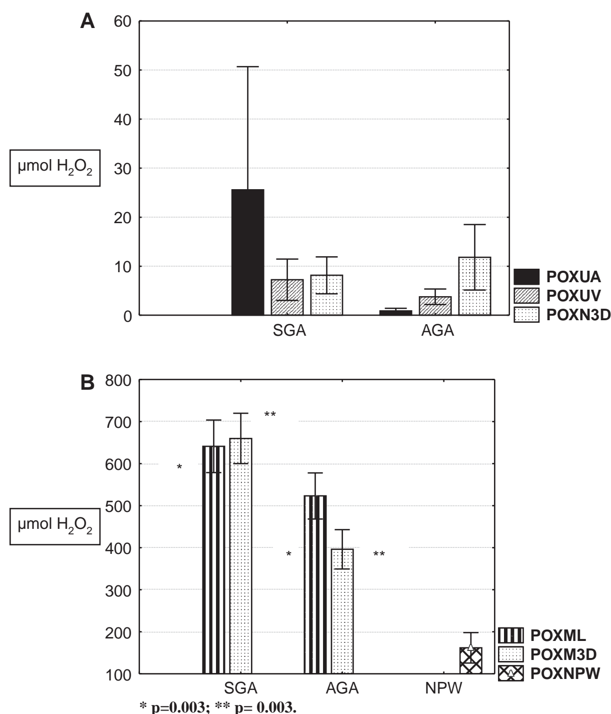

Figure 2. Peroxide values in the blood of newborn babies and their mothers. (A) Peroxide values in the blood of newborns. (B) Peroxide values in the peripheral blood of mothers. SGA, small for gestational age; AGA, appropriate for gestational age; NPW, non-pregnant women; POXUA, POX in umbilical artery; POXUV, POX in umbilical vein; POXN3D, POX in newborns on 3rd day of life; POXML, POX in mother at delivery; POXM3D, POX in mother on 3rd day after delivery; POXNPW, POX in non-pregnant women. Values are given as mean±SE.

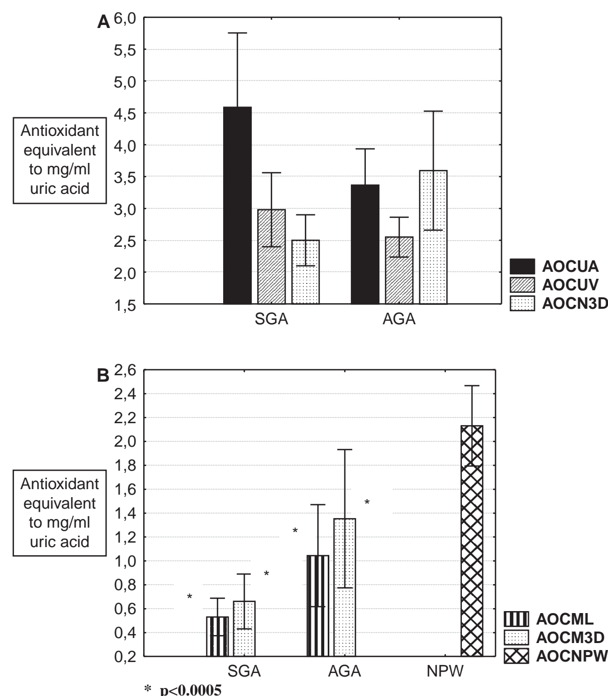

Figure 3. Serum antioxidant capacity of newborn babies and their mothers. (A) Serum antioxidant capacity of the newborns. (B) Serum antioxidant capacity of mothers. SGA, small for gestational age; AGA, appropriate for gestational age; AOCUA, AOC in umbilical artery; AOCUV, AOC in umbilical vein; AOCN3D, AOC in newborns on 3rd day of life; AOCML, total antioxidant capacity of mothers at delivery; AOCM3D, antioxidant capacity of mothers on 3rd day after delivery; AOCNPW, AOC of non-pregnant women. Values are given as mean±SE.

The results of the serum antioxidant capacity determinations are given in Figure 3. Capacity of antioxidants was higher in the umbilical cord arteries than in the veins for both AGA and SGA newborns, while the highest values of serum antioxidants were determined in the umbilical arteries of SGA newborns without significance (Figure 3A). Afterwards, the blood of SGA newborns gave lower antioxidant values in the blood on the 3rd day after birth than the AGA newborns.

Mothers of both SGA and AGA children had significantly lower antioxidant capacity than non-pregnant women ($p < 0.0005$). Moreover, SGA mothers had lower levels of serum antioxidant at delivery and on the 3rd day after delivery than did mothers of AGA newborns, without significance (Figure 3B).

The levels of the serum antioxidant capacity in umbilical artery, umbilical vein and in peripheral blood of newborns of both groups on the 3rd day of life were higher than in their mothers (SGA: ML/UA $p = 0.005$; ML/UV $p = 0.004$; M3D/N3D $p = 0.003$; AGA: ML/UA $p = 0.028$; ML/UV $p = 0.002$; M3D/N3D $p = 0.009$). If we compared them with non-pregnant women, newborns had slightly increased capacities of antioxidants, but only in the umbilical cord arteries ($p = 0.048$).

Our results demonstrated significantly positive correlation between certain biomarkers within groups, such as mother's MDA level at delivery and MDA in the umbilical vein, the level of MDA between the umbilical vein and the artery ($p < 0.0005$), while the level of POX did not show any significance in the same variables.

Comparison of the values obtained for mothers and the newborns for different parameters of oxidative stress and lipid peroxidation analysed in our study revealed positive correlations for MDA between babies and their mothers for both SGA and AGA groups, as presented in Table II. Moreover, the MDA values determined for mothers at the birth were in positive correlation with the values determined on the third day after delivery. Similar positive correlations were also found for the serum peroxides and the antioxidant capacities of sera when the values obtained from the blood of mothers during delivery were correlated with the values obtained on the 3rd day after delivery.

Finally, it should be mentioned that results obtained on placenta tissue samples revealed strong immunopositivity for the HNE-protein adducts in stromal cells in almost two thirds (62.5%) of the SGA samples analysed, while such a prominent presence of the HNE was noticed in only 12% of the AGA placental samples

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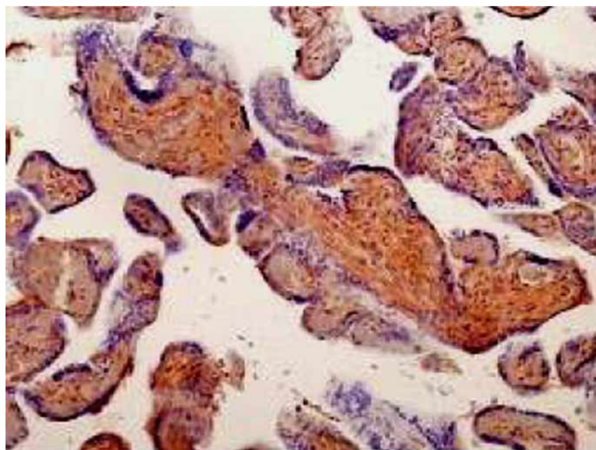
Table II. Correlations between oxidative stress biomarkers (Spearman's rank correlation).

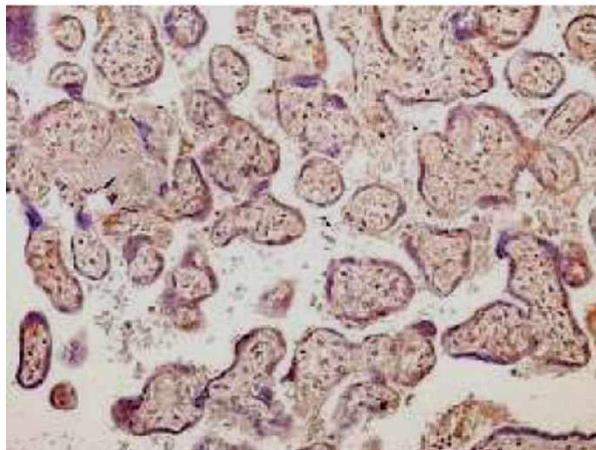
Variable–sample	Newborns, AGA		Newborns, SGA	
	Correlation coefficient	p	Correlation coefficient	p
MDA—mother at delivery: umbilical vein	0.611	<0.0005	0.577	0.001
MDA—mother at delivery: umbilical artery	0.684	0.0005	0.545	0.002
MDA—umbilical vein: umbilical artery	0.634	0.0005	0.648	0.0005
MDA—mother at delivery: Mother 3rd day	0.599	0.001	0.053	0.051
POX—mother at delivery: mother 3rd day	0.437	0.016	0.615	0.011
AOC—mother at delivery: mother 3rd day	0.614	<0.0005	0.879	<0.0005

MDA, malondialdehyde (HPLC determined); POX, total serum peroxides (enzymatic assay); AOC, serum antioxidant capacity (enzymatic assay).

($p < 0.01$, Table III). However, trophoblasts were mostly negative or slightly positive for HNE in both groups, while in the case of AGA group placental stroma was also mostly negative or slightly positive (Figure 4). According to these findings we can assume that placenta and in particular its stromal part may play a pathophysiological role in SGA in respect to the increased lipid peroxidation.



SGA



AGA

Figure 4. Immunohistochemistry for HNE-protein adducts in placental tissue (magnification 200 ×). Stromal cells in the SGA show strong immunopositivity for the HNE-protein adducts (brown colour in the central part of each tissue fragment—i.e. placental villi), while stroma of the villi of the AGA placenta show no positivity (light yellow staining). Trophoblast cells of the outer layer of the villi (surrounding the stroma) is negative, i.e. blue coloured in both cases.

Discussion

Our study has shown onset of oxidative stress during birth in both infants and their mothers, as was observed already by other researchers using different analytical methods [12–16]. Although such an oxidative stress might be considered even as physiological in the case of normal birth, we found it more pronounced and longer lasting in SGA mothers and babies. The SGA mothers at delivery had higher levels of MDA than AGA mothers at delivery and than NPW. Their higher values of peroxides (POX) as well as lower levels of serum antioxidants (AOC) support the assumption that oxidative stress might indeed be related to the SGA pregnancy and is associated both with the stress on mothers and babies, in particular mothers.

Similarly, higher levels of MDA in umbilical artery and umbilical vein of SGA newborns show that these babies suffered from lipid peroxidation and more pronounced oxidative stress than the AGA newborns. Gupta et al. [21] found increased MDA levels in the umbilical vein of SGA newborns born by undernourished mothers. Sridhar et al. [37] investigated mothers with some disease (eclampsia, pre-eclampsia, heart disease) and found increased MDA level in the SGA group. Arguelles at al. [38] found higher concentrations of lipid hydroperoxides and carbonyl groups, as well as lower levels of antioxidant capacity

Table III. Immunohistochemical detection of the HNE-protein adducts in placental tissue of the SGA and AGA mothers.

Intensity of the HNE-immuno positivity	SGA Stromal cells	SGA Trophoblast cells	AGA Stromal cells	AGA Trophoblast cells
Negative (0)	0%	60%	20%	40%
Weak (1)	37.50%	20%	60%	32%
Medium (2)	0%	20%	12%	28%
Strong (3)	62.50%	0%	8%	0%

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in umbilical cord blood in comparison to the maternal blood. They also found positive correlations between maternal blood and cord blood in biomarkers of oxidative stress, as well as negative correlation of peroxides and AOC. We did not found higher levels of peroxides in umbilical cord, neither in umbilical artery nor in umbilical vein, while the POX assay we used revealed peroxide levels much below the values determined in the blood of mothers and even non-pregnant women, indicating that in newborns serum peroxides are rapidly metabolized. It should also be said that Arguelles at al. [38] took venous blood from mothers during the ante partum period, while we took the blood samples immediately after delivery, with the umbilical cord still uncut. Perhaps that may be another reason for higher levels of peroxides in mothers at delivery in our study.

Kim et al. [39] found an inverse association between maternal urinary 8-hydroxydeoxyguanosine and MDA and the birth weight of their newborns. Our findings of high levels of MDA in blood of mothers at delivery in the SGA group support these findings, suggesting higher oxidative stress in the SGA group.

The blood in umbilical vein is a reflection of the mother-placenta complex relations. Our findings imply that oxidative stress was more expressed in mothers of SGA newborns which may be extended over placenta in additional oxidative stress of foetus and newborns. It could be explained by failure of the placenta's protective role, although the antioxidant defense of placenta was not well studied. The assumption that maternal stress is essential in pathogenesis of SGA is supported by the results of all parameters we analysed, namely not only that MDA and POX were higher, while AOC was lower in the SGA mothers, but they also did not change during the 3 days after labour.

Similar results were observed also for SGA newborns, but not as obvious as in the case of mothers. It is likely that their status was after birth indirectly influenced by mothers not over maternal blood but over breastfeeding. Several authors have written about nutrition and oxidative stress, both in mothers and mostly in premature newborns [19,40–42]. Therefore, it is likely that breastfeeding influenced postnatal oxidative stress in babies, supporting attenuation of oxidative stress in AGA babies. This might help us to explain higher MDA in SGA newborns in comparison to the AGA babies, however it was certainly not the cause of increased oxidative stress in SGA, especially not during labour.

There was a significant positive correlation between the maternal MDA at delivery and the MDA of umbilical artery and vein in SGA group, but the newborns of the AGA group also showed the same correlation. Moreover, a positive correlation was found between the umbilical vein and the umbilical artery of both groups. In light of that, a question of the protective placental role against MDA secretion or excretion in fetoplacental circulation in the SGA group should be considered [16,43]. Also, oxidative stress could develop in some conditions like hypoglycemia and hypoxia. Both conditions can be found in the foetus with IUGR and might possibly progress with the child development.

In our study peroxides were significantly higher in mothers, while their levels were low in arterial and venous cord blood in our study. Steinerova et al. [18] found a significantly higher peroxide level in mothers after delivery, while Rogers et al. [16] found lower peroxides levels in cord blood. Thus, our findings confirm both these studies. It is known that the process of labour is associated with the interrupted substrate flow from the placenta to the foetus and vice versa. That should result in intermittent phases of ischemia-reperfusion and free radical production. Consequently, peroxides could rise initially and decline subsequently. Peroxides are mediators of lipid peroxidation and their activity could lead to the spread of peroxidation and generation of the end-products of lipid peroxidation, such as reactive aldehydes as MDA and HNE that may have various toxic and growth regulating activities [6,7]. Rogers et al. [44] considered a possibility of differential placental handling of peroxides and MDA and potentially different origins of these metabolites. Therefore, various parameters interacting in complex metabolic mechanisms could affect oxidative metabolism of lipids and lipid peroxidation products during labour. The peroxide levels from umbilical cord (artery and vein) and those of newborns were significantly lower than in mothers and NPW. That is in compliance with Rogers et al. [44]. The fact that mothers had very high peroxide levels, while the umbilical blood, both arterial and venous had negligible amounts of peroxides suggests not only the important role of the placenta in regulation of the oxidative status/stress during labour but also potential relevance of systemic oxidative stress and lipid peroxidation for pathophysiology of birth. We assume that low levels of peroxides in newborns in the 3rd day of life in comparison to high levels of MDA indicates that newborns either utilize peroxides for metabolic purposes or that peroxides are toxic to newborns and are therefore metabolized rapidly by antioxidants in newborns to avoid their possible toxicity. In favour of this possibility were findings of higher AOC levels in newborns vs mothers and even NPW. We did not observe lower levels of AOC in the umbilical cord and newborns, as described by some authors, which may be due to different analytical assays used [12–15].

In light of our findings, the conclusion may be drawn that both the newborns and the mothers of the SGA group suffer from oxidative stress and lipid peroxidation. The process of oxidative stress was more expressed in mothers of the SGA newborns

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not only during the labour but also on the 3rd day after labour. Therefore, the question has to be asked, to what extent stress may be generated by diet, psychological problems, care for their children, establishment of lactation and lactation itself.

Because recent study revealed that prominent oxidative stress in young people born as SGA may originate from enhanced oxidative stress in SGA mothers and SGA newborns [29], while enhanced markers of oxidative stress had been found in children with cerebral palsy [45], the question could be asked about the cause of increased oxidative stress in the SGA group. Since there are no obvious, pathological reasons for prenatal as well as for postnatal oxidative stress, we can assume that the mediators of oxidative stress which might affect proliferation, differentiation and apoptosis, such as lipid peroxidation products, might be involved in SGA. Increased MDA values in SGA mothers and babies together with the immunohistochemical results showing abundant HNE presence in stromal cells of placental tissue of the SGA children obtained support this assumption. It has yet to be evaluated in further studies if prenatal oxidative stress and lipid peroxidation of SGA were due to excess in ROS and/or reactive aldehydes in placental circulation and in the placenta itself affecting both mothers and babies. However, since HNE is known as a second toxic messenger of free radicals that also acts as a growth regulating factor interfering with the humoral growth factors present in serum [6–10,46,47] it is probable that such unfavourable circumstances would not allow optimal intrauterine growth.

Our study indicates that increased oxidative stress of SGA newborns and mothers could indeed alter oxidative homeostasis of babies in their very delicate period of development that might become even worse by time influence on them. Therefore, further follow-up studies of SGA children might help us to understand the pathophysiology of oxidative stress in SGA, which is necessary to be able to help these mothers and their children.

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