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Evaluation of Oxidative Stress Markers after Vaginal Delivery or Caesarean Section

ZSUZSANNA HRACSKO[1], ZSOLT SAFAR[2], HAJNALKA ORVOS[3], ZOLTAN NOVAK[4], ATTILA PAL[3] and ILONA S. VARGA[1]

[1]*Department of Biochemistry and Molecular Biology, University of Szeged, H-6701 Szeged;*
[2]*Department of Biotechnology University of Szeged, H-6726 Szeged;*
[3]*Department of Obstetrics and Gynecology University of Szeged, H-6701 Szeged;*
[4]*Department of Pediatrics, University of Szeged, H-6720 Szeged, Hungary*

Abstract. *Background: The effects of vaginal delivery (VD) and of cesarean section (CS) on the markers of oxidative stress were investigated. Materials and Methods: Umbilical blood samples were analyzed from 74 full-term neonates, 46 born via VD, 28 via elective CS. The level of lipid peroxidation (LP), protein and DNA damage and the antioxidant status were compared. Results: Differences between CS and VD groups were generally non-significant for oxidative markers, except for the GSH concentrations (VD: 4.18 vs. CS: 2.77 μM/mg protein x 10^{-3}; $p<0.05$). LP was significantly higher in the CS group (0.078 vs. 0.042 nM MDA/mg protein; $p<0.05$). The level of carbonyl proteins was high in the VD group and significantly lower in the elective CS group (9.5 vs. 8.1 mM/mg protein x 10^{-4}; $p<0.05$). We found 0.78% more strand breaks in elective CS group than in VD group. Conclusion: CS does not have an advantage over VD with respect to oxidative stress.*

The mode of delivery may have a considerable effect on the state and health of the newborn. In recent years, the proportion of deliveries carried out using Caesarean section (CS) has risen substantially around the world. CS is a surgical intervention with potential hazards for both mother and child. It also uses more resources than normal vaginal delivery (VD) (1). The opinions of obstetrician-gynecologists regarding normal spontaneous VD and CS are highly contradictory. Both the mother and fetus may be benefited or harmed by CS. It is a major abdominal surgery. When a CS is necessary, it can be a life-saving technique for both mother and infant, but the absence of labour could increase the risk of breathing problems and other complications. CS can delay the opportunity for early mother-newborn interaction, breast-feeding and the establishment of family bonds.

Emergency CS is performed when the state of the mother and/or the baby is critical. When CS is elective, there is no emergency situation (breech presentation, dystocia). There is still no agreement as to which method is more suitable for avoiding unfavourable consequences for the baby and the mother. We have approached this question from a consideration of oxidative stress.

The living organism is constantly exposed to oxygen. As a consequence of this activity, highly reactive molecules are produced. These interact with other molecules within the cell, which can cause oxidative damage to proteins, membranes and genes. This damage has been implicated in the cause of certain diseases, including neonatal diseases. The antioxidant system defends against free radicals. Under normal conditions, reactive oxygen species are cleared from the cell by the action of antioxidant molecules and enzymes. Oxidative stress is an imbalance between prooxidants and antioxidants, with the former prevailing. The main damage to cells results from the stress-induced alteration of macromolecules, such as polyunsaturated fatty acids in membrane lipids, essential proteins, and DNA.

The results of previous studies display great differences. Some authors claim that CS is advantageous in order to avoid oxidative stress. Umbilical arterial lipids are more susceptible to peroxidation than umbilical venous lipids, indicating a high level of oxidative stress in the fetal circulation irrespective of the mode of delivery (2). Elective CS does not appear to be superior to vaginal birth (3). Others declare that CS may cause a deficiency of antioxidant defence in the human newborn (4).

We set out to determine a wide range of parameters relating to the oxidative status. In the present study we investigated the effects of VD and of elective CS on

Correspondence to: Zsuzsanna Hracsko, Department of Biochemistry and Molecular Biology, University of Szeged, H-6701 Szeged, P.O. Box 533, Hungary. Tel: +36 62 544 543 Fax: +36 62 544 887, e-mail: hracsko@bio.u-szeged.hu

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antioxidant status (the level of glutathione (GSH) and ferric reducing ability of the plasma (FRAP)), the activities of antioxidative enzymes (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GP)) and the amount of lipid peroxidation (LP), protein and DNA damage in the umbilical cord blood. Lipid peroxides and carbonyl proteins are typical products of an oxidative milieu, but DNA is also the target of free radical attack. Protection of the DNA against high levels of oxidants is fundamental to avoid strand breaks and mutations, but physiological oxidative stress is indispensable in stimulating the expressions of the various antioxidant enzymes.

Materials and Methods

Umbilical cord blood samples were obtained from the Department of Obstetrics and Gynecology of the Medical University of Szeged, Hungary. The Ethics Committee of the Department of Obstetrics and Gynecology, Faculty of Medicine, University of Szeged approved the study protocol. Blood was taken from the umbilical vein. Seventy-four full-term mature neonates of both sexes, born between weeks 37 and 40 of gestation were included; 46 were born by VD, and 28 delivered by elective CS. The birth weight was 3750 g±850 g. All of the cases were free from complications and there was no pre-eclampsia. The Apgar score after five minutes was 8-10. The mothers received complete pregnancy care. All subjects were singleton pregnancies selected from the general obstetric population with a minimum gestational age of 37 weeks. They were normotensive and nonobese. Patients with a history of renal disease, diabetes, or significant perinatal complications were excluded. There were no perinatal asphyxia, no congenital anomalies or special morbidities.

Blood coagulation was inhibited by EDTA. The blood samples were centrifuged at 1200 rpm for 10 min and the plasma and the buffy coat were removed. The red blood cell (RBC) phase was washed twice with 2 volumes of isotonic saline solution at pH 7. RBCs were haemolysed by adding distilled water in a ratio 1:9. Except for determining SOD activity, portions of the haemolysates were used directly.

Determination of SOD activity. Before the determination of SOD activity, the haemolysates were treated with ethanol:chloroform (2:1) to remove haemoglobin from the samples, then centrifuged at 5000 rpm. The supernatants were used for SOD activity determinations *via* inhibition of the epinephrine-adrenochrome transformation (5, 6). The control sample contained 2.9 ml of 0.05 M carbonate buffer (pH 10.2, warmed to 37˚C) and 0.1 ml of epinephrine (16.5 mg/10 ml 0.1 N HCl). The absorbance was measured at 480 nm for 3 min after a one-minute delay. We determined the rate of autooxidation of epinephrine (ΔA at 480 nm/min). To measure the inhibition of autooxidation by SOD a mixture containing: 2.875 ml of the carbonate buffer, 0.025 ml supernatant and 0.1 ml epinephrine was used. Spectrophotometric measurement was carried out at 480 nm (Thermospectronic Biomate5). The results were expressed in U/mg protein.

Determination of CAT activity. In order to determine the CAT activity, erythrocyte haemolysates (100-fold dilution) were used. The blank sample was 3 ml of phosphate buffer (pH 7.0) in a quartz cuvette. The incubation mixture contained: 2 ml of the phosphate buffer (warmed to 37˚C), 5 μl of haemolysate and 1 ml of H_2O_2 solution. CAT activity was measured spectrophotometrically at 240 nm. The results were expressed in Bergmeyer units (BU). One BU is the amount of CAT that decomposes 1000 mg H_2O_2/min (7).

Determination of GP activity. GP was also determined spectrophotometrically using cumene hydroperoxide and GSH as substrates, at 412 nm. All samples contained 0.1 ml of haemolysates and 0.7 ml of 0.05 M Tris-HCl buffer (pH 7.6) were preincubated for 10 min at 37˚C. To the control sample, 0.1 ml of buffered GSH solution was added, while the experimental samples were fed with 0.1 ml of GSH solution and 0.1 ml of buffered 0.05% cumene hydroperoxide. The samples were then incubated for 10 min at 37˚C. After 10 minutes 15% trichloroacetic acid (TCA) was added to all tubes, and 0.1 ml cumene hydroperoxide solution was added to the control tube. The precipitated protein was spun down and 1 ml supernatant samples were taken for determination of the remaining GSH. These were treated with 2 ml of 0.4 M Tris-HCl buffer, and 5,5 dithio*bis*-2-nitrobenzoic acid (DTNB) (8).

GSH and protein determinations. The GSH in the plasma and RBCs was measured by using Ellman's reagent. Proteins were precipitated with 5% TCA in order to exclude protein-linked SH groups in the measurement (9).

The quantity of total protein content was determined with the Folin reagent (10).

Carbonyl proteins are oxidatively damaged proteins. The carbonyl protein content was determined by using 2,4-dinitrophenylhydrazine (DNPH). DNA also contains carbonyl groups, so it was removed with 10% streptomycin-sulphate. Samples were incubated with DNPH at 37˚C. After one hour, 0.5 ml TCA was added. Samples were then centrifuged at 10000 rpm for 5 min. Pellets were washed three times with ethanol:ethyl-acetate (1:1), resuspended in 2 N HCL (pH 2) and incubated at 37AC for 15 min. After centrifugation, the absorbance of supernatants was read at 375 nm (11).

FRAP assay. The antioxidant capacity of the plasma was determined with the FRAP assay, a simple test measuring the ferric reducing ability of plasma, using 2,4,6-tripyridyl-s-triazine. Ferric to ferrous ion reduction at low pH causes a coloured ferrous-tripyridyltriazine complex to form. FRAP values are obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing ferrous ions at a known concentration (12).

Determination of LP and DNA damage. The LP of RBCs was determined by using the thiobarbituric acid (TBA) method (13), which reveals the level of total TBA-reactive substances. Calibration was performed with malonyl dialdehyde (MDA). A volume of 2.7 ml of TBA reagent solution (a mixture of 0.375% TBA, 0.25 M HCl, 15% TCA) was added to 0.3 ml of the haemolysed blood sample, the components were mixed vigorously and the mixture was heated for 15 min in a boiling water- bath. Subsequently, the samples were cooled in ice-cold water and centrifuged at 10000 rpm for 10 min; the absorbance of the supernatant at 532 nm was measured spectrophotometrically to calculate concentrations.

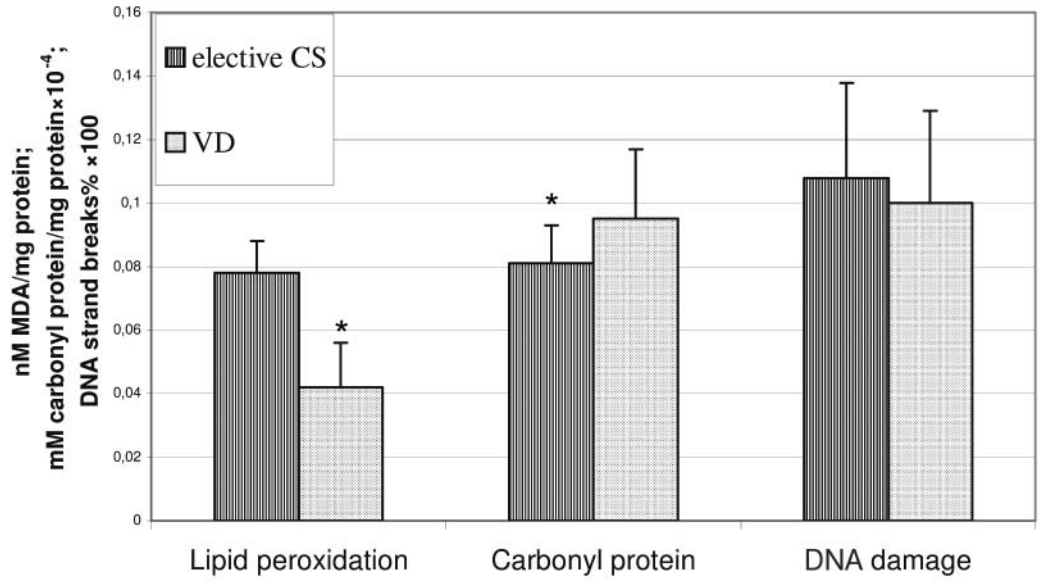

Figure 1. *Oxidative damage after Caesarean section (CS) and vaginal delivery (VD). *p<0.05.*

In order to detect oxidative DNA damage, DNA from the blood was purified (14). The result of the preparation was checked by means of agarose gel electrophoresis; the amount of DNA was determined by measuring the optical density. Oxidative DNA damage was detected by fluorimetric method (15). Fluorescence analysis was carried out with dual-channel modulated fluorimeter (Hansatech, King's Lynn, UK) in the emission region of 590 nm.

Statistical analysis of the data was performed using Student's *t*-test. $P<0.05$ was accepted as statistically significant. The Shapiro-Wilks test was applied to confirm the normality of the values.

Table I. *Differences between the antioxidant status after caesarean section (CS) or vaginal delivery (VD).*

	CS (n=28)	VD (n=46)	Significance *p*
μM GSH/mg protein x 10^{-3}	2.77±0.71	4.18±0.48	0.027
SOD U/mg protein	1.12±0.25	1.49±0.28	0.061
CAT BU/mg protein x 10^{-4}	7.2±2.19	8.13±0.95	0.059
GP U/mg protein x 10^{-3}	1.73±0.42	1.91±0.33	0.071
FRAP μM/L	306.4±25.4	323.5±18.3	0.055

Results

The Table I and Figure 1 present the results of the measurements. Differences between elective CS and VD groups were generally non-significant. The GSH concentration was significantly higher in the VD group ($p<0.05$). LP was higher in the CS group than in the VD group. The level of carbonyl proteins was significantly lower in the elective CS group ($p<0.05$). The DNA damage tests did not reveal a remarkable difference between the elective CS and VD groups (0.78% more strand breaks in the elective CS group).

Discussion

Oxidative stress is a physiological event in the fetal-to-neonatal transition. Our paper studies the influence of the mode of delivery on oxidative stress in the neonatal cord blood. The degree of oxidative damage and the antioxidant status depend on many factors. A low pH induces lipid peroxidation in the blood. When the pH<7.2 the hypoxic state results in an increase of malonyl dialdehyde concentration (16, 17). The samples involved in our study were included only if they had a normal pH (pH 7.2-7.4). Neonates with anomalies or special morbidities were also excluded in order to avoid any such influence on the oxidative stress. In agreement with a recent study (2), we found that the likelihood of CS is being advantageous in avoiding oxidative stress is questionable.

The antioxidant enzyme activities were higher in the VD group, although the differences were non-significant, but the level of GSH was significantly higher in the VD group. Other authors also suggest that the glutathione levels in venous and arterial umbilical samples were higher after vaginal delivery as compared to Caesarean section (18). A recent study claims that, the nonenzymatic antioxidant reserve is higher in infants delivered by VD (19).

The level of LP in the VD cases was very low, even lower than in the elective CS group. The integrity of the lipid membranes of RBCs is essential. Without an intact membrane structure, erythrocytes are unable to complete their task of transfer. During the process of birth, neonates face a high dosage of oxygen radicals. Although the concentration of GSH and the higher enzyme activities suggest a higher level of oxidative stress in VD group, neonates born *via* VD were less susceptible to this stress than those delivered by CS: the VD group exhibited lower LP and DNA damage. On the whole, the extent of oxidative damage during the process of elective CS differed only slightly and non-significantly from that in the VD group. There are many aspects, which must be considered before choosing elective CS instead of VD. The steadily increasing global rate of Caesarean deliveries has become one of the most debated topics in maternity care (20). We conclude that the mode of delivery does not have a serious effect on the level of free radical damage if there is no emergency situation. The elective CS does not have an advantage over VD with respect to oxidative stress.

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