Post-delivery oxidative stress in women with preeclampsia or IUGR

Patricia Kressig[1], Ernst Beinder[1], Horst Schweer[2], Roland Zimmermann[1] and Ursula von Mandach[1],*

[1] Department of Obstetrics, Zurich University Hospital, Zurich, Switzerland

[2] Department of Pediatrics, Philipps University Marburg, Germany

Abstract

Aim: To compare oxidative stress in patients with pre-eclampsia (PE) or intrauterine growth restriction (IUGR) vs. normal pregnancy (controls) during 48 h after delivery. **Study design:** Women with singleton pregnancies were recruited immediately after delivery (gestational age >26.0 weeks). Women with PE or IUGR were matched with healthy controls by age, BMI, gestational age and delivery mode. Venous blood samples and urine samples were tested for oxidative stress products 24 h and 48 h after delivery. **Results:** Plasma malondialdehyde (MDA) concentration 24 h after delivery was significantly higher in subjects with PE or IUGR (3.41 ± 1.14 μmol/L, n=20) than in controls (2.91 ± 0.82 μmol/L, n=38) (P=0.04). Urine iPF$_{2\alpha}$-VI declined from 24 to 48 h after delivery significantly in controls (P=0.006) and not in subjects with PE or IUGR (P=0.71). **Conclusion**: Of the markers tested only MDA is indicating higher oxidative stress in women with PE/IUGR than in normal pregnancy and only at 24 h after delivery. No consistent pattern of change in the oxidative stress markers exists between 24–48 h after delivery.

Keywords: Gestational hypertension; isoprostanes; IUGR; malondialdehyde; oxidative stress; PE; pregnancy.

Introduction

Oxidative stress is defined as an imbalance between free radical damage and antioxidant protection, caused by the presence of free radicals or radical-generating agents. Oxidative damage belongs to biological substances such as lipids, DNA and proteins [6]. Lipid peroxidation products in plasma and urine initiated in placenta through an exaggerated placental production of superoxide anions (free radicals) are therefore frequently used biomarkers for oxidative stress.

Pregnancy itself is a condition of increased oxidative stress due to increased mitochondrial activity and reduced scavenging potential [32]. Elevated levels of oxidative stress status in pregnancy were shown in many studies [24]. This status is aggravated in pregnancies with IUGR or preeclampsia [29]. Preeclampsia accounts for more than 40% of premature deliveries and is also the most important cause for maternal mortality and morbidity in developing countries. The maternal endothelium is the ultimate target leading finally to preeclampsia where free radicals evoking endothelial-cell activation are obviously promoters of maternal vascular malfunction.

Oxidative stress may be responsible not only for pre-eclampsia or IUGR but also for other endothelial cell generated dysfunctions such as atherosclerosis and cardiovascular diseases [7, 19, 27]. The fetus as well as the mother may therefore develop cardiovasuclar problems after pregnancies with elevated oxidative stress, especially if oxidative stress persists post-partum [18]. Indeed, in a study measuring lipid peroxidation products elevated levels have been found before and also after delivery in maternal serum from women with PE compared to controls [17]. We hypothesize that pregnancies associated with elevated oxidative stress such as PE and IUGR have elevated oxidative stress parameters in blood and urine during the first 48 h after delivery. The objective of this study was therefore to measure oxidative stress markers within the first postpartal 48 h. We focussed thereby on two markers reflecting oxidative stress generated from presumably different mechanisms: Malondialdehyde (MDA) in plasma is a product of the reaction of peroxynitrite with lipids and prostanoids in urine are generated by non-enzymatic peroxidation of archidonic acid cleaved into the blood and excreted in the urine where it reflects oxidative stress *in vivo* [13, 23].

Materials and methods

Subjects and protocol

Women who delivered >26.0 gestational weeks at the Department of Obstetrics, University Hospital of Zurich, were consec-

*Corresponding author
Prof. Dr. Ursula von Mandach
Department of Obstetrics
Zurich University Hospital
Frauenklinikstr. 10
CH–8091 Zurich
Switzerland
Tel.: +41 44 255 51 36
Fax: +41 44 255 44 30
E-mail: ursula.vonmandach@usz.ch

utively recruited during 12 months. Women with normal pregnancy (controls) were matched by age, BMI, gestational age, and delivery mode 2:1 to women with high oxidative stress associated pregnancy, i.e., with preeclampsia (PE) or intrauterine growth restriction (IUGR). Exclusion criteria were: multiple pregnancy, neonatal infection, amnion infection syndrome, CRP ≥ 5 mg/L, BMI ≥ 30 kg/m², gestational diabetes, diabetes mellitus, cardiovascular diseases, neoplasia, hepatitis, HIV infection; nicotine, alcohol and drug abuse. All volunteers gave written informed consent to participate in this study. BMI was calculated from the body weight measured between the 6th and 8th week of gestation (representing the pre-pregnancy weight). Demographic data are taken from the patient data files. Gestational age was calculated from the first day of the last menstrual period and first trimester ultrasound; dates were corrected to the ultrasound data where appropriate. PE was defined as gestational hypertension with a diastolic pressure ≥ 90 mmHg taken on two occasions 6 h apart and proteinuria ≥ 300 mg per 24 h and/or $\geq 2+$ on dipstick analysis of midstream (or catheter) urine specimen according to the criteria of the International Society for the Study of Hypertension in Pregnancy (ISSHP). IUGR was defined as gestational related sex-specific birth weight below the 10th percentile and abnormal Doppler examination [31].

On the first and on the second post-delivery day (24 and 48 h after delivery) always at 06:00 after overnight fast, 5 mL of venous blood were taken to analyze plasma malondialdehyde concentration. The minimal time between delivery and first blood sampling had to be 6 h. In addition, the subjects gave two morning urine samples, each collected between 00:00 a.m. and 06:00 a.m. which were used to measure creatinine and isoprostanes (8,12- *iso*-iPF$_{2\alpha}$-VI and iPF$_{2\alpha}$-VI).

Analytical methods

Malondialdehyde (MDA)   EDTA blood was separated by centrifugation at 4°C immediately after blood-withdrawal and plasma samples were stored at −20°C until analysis using the Lipid Peroxidation Assay Kit (Cat. No. 437634) from Calbiochem (VWR International AG, 8953 Dietikon, Switzerland). The principle of this colorimetric assay is the reaction of a chromogenic reagent with MDA at 45°C. One molecule of MDA undergoes condensation with two molecules of the reagent to yield a stable chromophore with maximal absorbance at 586 nm. The detection limit was 0.1 μM MDA and the interassay variation was $<5\%$.

8,12-*iso*-iPF$_{2\alpha}$-VI and iPF$_{2\alpha}$-VI   Urine samples were snap-frozen immediately after collection and stored at −80°C until further analytical procedure. Urine (1 mL), [²H$_{11}$]-8,12- *iso*-iPF$_{2\alpha}$-VI and [²H$_{11}$]-iPF$_{2\alpha}$-VI (1 ng each) were acidified with formic acid to pH 2.5 and the isoprostanes were extracted twice with 3 mL of ethyl acetate-hexane (70:30, v/v). After evaporation of the solvent, acetonitrile (80 μL), pentafluorobenzyl bromide (7 μL) and N,N-diisopropylethylamine (25 μL) were added. The mixture was allowed to react at 40°C for 25 min. The dry sample was purified by TLC (developing solvent: ethyl acetate-methanol 100:3, v/v). The zone with an R$_F$ 0.2–0.33 was eluted with TLC ethylacetate (800 μL) and water (50 μL) was added. After centrifugation, the ethyl acetate phase was withdrawn, the solvent was evaporated and the isoprostanes derivatized with BSTFA (10 μL; 40°C, 1 h). A 1 μL aliquot of this solution was injected. The isoprostane concentrations in the prepared urine samples were determined using gas chromatography/tandem mass spectrometry (GC/MS/

MS). A Finnigan MAT TSQ700 tandem mass spectrometer equipped with a Varian 3400 gas chromatograph and a CTC A200S autosampler (Finnigan MAT, Bremen, Germany) was employed. Gas chromatography of prostanoid derivatives was carried out on J&W DB-1 (15 m×0.25 mm I.D., 0.25 μm film thickness) capillary column (MasCom, Bremen, Germany) in the splitless mode at an inlet pressure of 100 kPa. The oven temperature program for all prostanoids analyzed was as follows: an initial temperature of 100°C was held for 2 min, and then increased at 30°/min to 280°C and at 5°/min to 310°C. This temperature was held for 2 min. The mass spectrometer conditions were: interface temperature 300°C, source temperature 150°C, methane CI gas pressure 50 Pa, electron energy 70 eV, emission current 0.4 mA, conversion dynode 15 kV, electron multiplier 2000 V and the collision cell pressure was 0.2 Pa. For iPF$_{2\alpha}$-VI the collision energy was 20 eV and the daughter ions used for quantification were − [P − (CH$_3$)$_2$Si=CH$_2$ − (CH$_3$)$_2$Si=O] $^-$ (m/z 569.3 → m/z 423.3 for the endogenous iPF$_{2\alpha}$-VI, m/z 573.3 → m/z 434.2 for the internal standard). For 8,12-iso-iPF$_{2\alpha}$-VI the parent ions of the characteristic fragment C$_6$H$_7$O$_2$$^-$ (m/z 111.0) were used: m/z 569.3 for the endogenous 8,12-iso-iPF$_{2\alpha}$-VI and m/z 580.4 for the deuterated internal standard. Collision energy was 28 eV. The urinary isoprostane, iso-iPF$_{2\alpha}$-VI and 8,12- *iso*-iPF$_{2\alpha}$-VI levels were corrected for creatinine values to eliminate influences of renal function on the result.

Isoprostanes   Urine (100 μL) was diluted with 200 μL water and tetradeuterated [²H$_4$]-PGF$_{2\alpha}$ (1 ng in 10 μL ethyl acetate) was added. Sample cleanup and derivatization was as described for 8,12-iso-iPF$_{2\alpha}$-VI and iPF$_{2\alpha}$-VI except TLC: developing solvent was ethyl acetate-hexane 90:10, v/v) and a broad zone (R$_F$ 0.03–0.39) was eluted with the TLC developing solvent (1 mL). GC/MS/MS parameters were as depicted for 8,12-*iso*-iPF$_{2\alpha}$-VI and iPF$_{2\alpha}$-VI, but the collision energy was 12 eV and the daughter ions used for quantification were [P − 3(CH$_3$)$_3$SiOH]$^-$ (m/z 569.3→m/z 299.3 for the endogenous isoprostanes and m/z 573.3 → m/z 303.2 for the tetradeuterated internal standard).

Statistics

Statistical analysis was performed using StatView for Windows, version 5.0.1 (SAS Institute Inc., © 1992–1998, www.statview. com). A power calculation considering a mean±SD malondialdehyde concentration of 2.9±0.4 μmol/L gave a power of 0.80 for n=20 per group. Mean±SD and the median were calculated. Graphical data are presented with mean±SE. The Kolmogorov-Smirnov test was used for normality, and the Wilcoxon signed rank test as well as the paired *t*-test of log values to detect longitudinal patterns. Controls vs. PE/IUGR differences were examined using the Mann-Whitney *U*-test as well as the unpaired two-tailed *t*-tests of log values or the Fisher's exact test, respectively. Group interaction with other parameters was tested using analysis of variance (ANOVA). Correlations between two variables were tested by multiple regression. A P-value <0.05 was considered significant in all tests.

Results

Sixty women were recruited; two women were excluded because of missing values, resulting in a total of 58

women (n = 20 with PE, IUGR, and n = 38 controls). The groups were similar with regard to age, BMI, race, and delivery mode, but different with regard to preeclampsia, birth or placental weight; seventeen newborns of the PE/IUGR group had a birth weight below the 5th percentile (Table 1). No significant difference exists between demographic data of women with PE and such with IUGR (data not shown). Mean interval between delivery and blood (begin of urine) sampling for both groups was 20.2 h (so called 24 h time point) and 44.2 h after delivery (so called 48 h time point).

Malondialdehyde (MDA) plasma concentration was significantly elevated 24 h after delivery in women who had pregnancy with PE and/or IUGR (P = 0.04), whereas the levels of the other parameters were not significantly different either at 24 or at 48 h after delivery compared to controls (Table 2). In the whole collective, all parameters declined from 24 to 48 h after delivery. However, if groups were considered, only MDA and $iPF_{2\alpha}$-VI levels changed significantly between 24 and 48 h: differences between MDA plasma levels were significant in women with PE/IUGR but not in controls (P = 0.04) (Figure 1), and

$iPF_{2\alpha}$-VI declined significantly from 24 to 48 h only in women with normal pregnancy (P = 0.006) but not in women with PE/IUGR (Figure 2). No intra-group differences in oxidative stress parameters were found if results from women with PE alone (n = 6) were compared to that from women with IUGR alone (n = 14). Multiple regression analysis did not show any additional influence of parity or delivery mode on the demonstrated differences.

Comment

We could show that malondialdehyde plasma concentration was significantly elevated in women with PE or IUGR 24 h after delivery and $iPF_{2\alpha}$-VI declined less from 24 to 48 h after delivery in comparison to healthy women with normal pregnancy.

Oxidative stress parameters vary significantly if they are not measured under standardized conditions. Known influencing factors are BMI, age, gestational age, CRP, food and drug intake as well as diurnal variations [4, 9, 16, 22]. We have considered these problems in our study

Table 1 Maternal characteristics at delivery.

	Controls (n = 38)	PE/IUGR (n = 20)	P-value
Age (years)	31.1 ± 5.2	31.9 ± 3.8	0.59
Height (m)	1.64 ± 0.06	1.64 ± 0.06	0.65
Weight (kg)	62.0 ± 10.1	61.0 ± 11.9	0.72
BMI (kg/m²)	23.11 ± 3.24	22.56 ± 4.37	0.59
Nulliparity (n)	18 (47.4)	15 (75)	0.05
Race: caucasian (n)	32 (84.2)	19 (95)	0.4
Delivery mode: c. section (n)	17 (44.7)	13 (65)	0.17
Gestational age (weeks)	37.2 ± 2.9	35.8 ± 3.5	0.11
Preeclampsia (n)	0 (0)	8 (40)	0.0005
Birth weight (g)	3134 ± 778	2104 ± 698	< 0.0001
Birth weight centile < 10 (n)	0 (0)	14 (70)	< 0.0001
Placental weight (g)	568.0 ± 184.9	369.4 ± 126.5	0.0001

Continuous values: mean ± SD; numeric values: n (%).
Differences between groups were tested by unpaired two-tailed t-test or two-tailed Fisher's exact test, respectivety.

Table 2 Oxidative stress parameters after delivery.

Parameter	Time post-delivery (h)	Controls (n = 38)	PE/IUGR (n = 20)	P-value
Malondialdehyde (μmol/L) (plasma)	24	2.91 ± 0.82	3.41 ± 1.14	S (0.04)
	48	2.81 ± 0.72	2.92 ± 0.49	NS (0.35)
Isoprostanes (ng/mg)* (urine)	24	71.85 ± 53.39	74.80 ± 42.29	NS (0.74)
	48	65.34 ± 34.63	76.37 ± 41.71	NS (0.27)
8,12-*iso*-$iPF_{2\alpha}$-VI (ng/mg)* (urine)	24	7.59 ± 4.91	5.29 ± 2.65	NS (0.31)
	48	6.50 ± 3.97	5.60 ± 4.22	NS (0.25)
$iPF_{2\alpha}$-VI (ng/mg)* (urine)	24	1.45 ± 0.80	1.43 ± 0.76	NS (0.54)
	48	1.13 ± 0.52	1.22 ± 0.53	NS (0.49)

Mean ± SD.
Differences of levels between groups were tested by unpaired two-tailed t-test of log values.
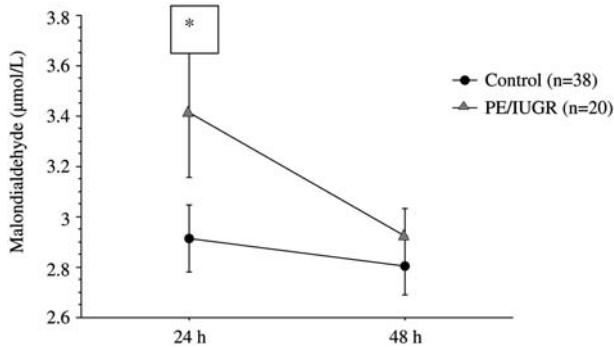*Corrected for creatinine.

Figure 1 Post-delivery malondialdehyde plasma concentrations. Women with PE/IUGR had significantly elevated malondialdehyde levels 24 h after delivery compared to women with normal pregnancy (*P = 0.04). Difference of levels between 24 and 48 h was significant in women with PE/IUGR (P = 0.04).
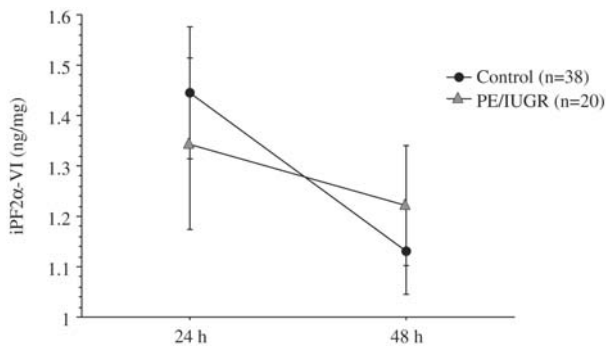

Figure 2 Post-delivery iPF$_{2\alpha}$-VI levels in urine. Women with PE/IUGR did not differ significantly from women with normal pregnancy. Difference of levels between 24 and 48 h was significant only in controls (P = 0.006).

design by using strong inclusion and exclusion criteria, by matching women with PE/IUGR with controls and by standardizing blood and urine sampling. Although matching by gestational age was performed as carefully as possible, we could not avoid that gestational age was insignificantly higher in the control group. However, this is a known problem in studies like the present one to select appropriate controls. Moreover, as other authors before [10, 29], we have not shown separate data for women with PE and for such with IUGR. This study design was applied because most of our women with PE have reduced intrauterine growth. Indeed, in our subgroup of women with PE (n = 8) there were two women with IUGR and five women with birth weights at percentiles > 10 ≤ 50.

Malondialdehyde (MDA) in plasma and prostanoids in urine corrected for creatinine are the most reliable markers for *in vivo* oxygen stress reflecting lipid peroxidation [2, 23]. MDA results from peroxidation of peroxynitrite with lipids [30]. In women with PE, MDA levels are elevated in placental tissue, in maternal plasma, in umbilical cord blood erythrocytes, as well as in the placental tissue [1, 5, 12, 13, 28]. It seems that oxygen damage in the placenta is mediated by increased MDA production followed by higher levels also in the maternal (and umbilical) circulation. The severity does correlate with both, the MDA concentration in the serum and that in the erythrocytes [26]. Accordingly, it was shown that the greater the level of lipid peroxidation, the greater the severity of PE [20]. Indeed, we have found in our study the highest MDA levels 24 h after delivery in women with PE (3.55 μmol/L; n = 8; data not shown). Controls did not change MDA levels after delivery; moreover, mean values were similar (2.91 μmol/L at 24 h and 2.81 μmol/L at 48 h) as that found in non-pregnant healthy, overnight fasted, controls from our recent study with the same MDA assay (2.88 μmol/L) [25].

Isoprostanes (or F$_2$-isoprostanes) are isomers of PGF$_{2\alpha}$ and free-radical catalyzed products of non-enzymatic lipid peroxidation of arachidonic acid. They are chemically stable, formed *in situ* at the side of the free-radical attack, and are cleaved from the plasma membrane and excreted in urine. Isoprostanes have been shown to be elevated in a number of disorders that are possibly associated with oxidative stress reflecting oxidative stress *in vivo* [23]. However, data about isoprostane levels in PE are controversial. F2-isoprostanes were elevated in plasma, but not in urine or in saliva [14, 15]. Otherwise, no difference in 8-iso-PGF$_{2\alpha}$ in plasma or urine [18] or in 8,12-*iso*-iPF$_{2\alpha}$-VI (urine only) was found in PE [22]. Significant difference in redox-related molecules has been found in placenta from pregnancies with PE and/or IUGR compared to controls [29]. Isoprostanes in amniotic fluid taken during amniocentesis at 15–18 weeks also predicted IUGR; it distinguished between normal pregnancies and those with SGA (birth weight below the 5th percentile) with a sensitivity of 100% and a specificity of 72% [11]. Moreover, in a study of 78 women, elevated and similar values of 8-iso-PGF$_{2\alpha}$ were found in maternal plasma of women with gestational hypertension or with IUGR compared to that with normal pregnancies, respectively [10]. Elevated maternal plasma levels of isoprostanes were also found by Harsem et al. 2006 [8]. Our results indicate no significant difference in urinary iPF$_{2\alpha}$-VI level between the high stress associated group and women with normal pregnancy. iPF$_{2\alpha}$-VI declined less from 24 to 48 h after delivery, but the pattern of change from 24 to 48 h was not consistent.

Oxidative stress in pregnancy may induce micro-damage to maternal arteries and might be a link to adverse maternal long-term morbidity such as atherosclerosis or cardiovascular diseases [7, 19, 27]. It was hypothesized that not only the mother is influenced by oxidative stress but that these damages can have long lasting impacts on the unborn baby (Barker's hypothesis of "Fetal Programming") [2, 21]. Until now only one *in vivo* study focussed on post-delivery lipid peroxidation products:

Thiobarbituric acid reactive substances are reported to be elevated in maternal serum before and also after delivery in women with PE compared to normal women [17].

In summary, we demonstrated that of the markers tested, only MDA indicated higher oxidative stress in women with PE/IUGR and only at 24 h after delivery. No consistent pattern of change in the oxidative stress markers exists between 24 to 48 h after delivery. In cases of PE/IUGR, MDA levels in maternal plasma, in contrast to urine isoprostane levels, may reflect oxidative damage in the placenta which spills-over to the maternal circulation from where it will be eliminated with time.

Acknowledgements

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