ERYTHROCYTE DAMAGE/AGING/REMOVAL ARE ENHANCED IN BOTH MOTHER AND FETUS, IN PREECLAMPSIA

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SUMMARY

The development of preeclampsia (PE) is linked to a failure of trophoblastic invasion of the spiral arteries. Within these arteries, the deposition of fibrinoid material and foam cells in PE may lead to a reduced blood flow, favouring the interaction between the surrounding cells. The longer exposure of red blood cell (RBC) to oxygen metabolites and proteases produced by inflammatory cells may account for RBC damage. We aimed to study if a continuous enhanced exposure to inflammatory activation products throughout a preeclamptic (PEc) gestation, would account for a higher RBC damage, which may compromise oxygen maternal-fetal exchange and, therefore, placental homeostasis and fetus development.

The study was performed in 42 healthy pregnant women and 44 preeclamptic pregnant women, and in their neonates. We evaluated maternal erythrocyte changes [RBC count, hemoglobin (Hb), hematocrit (Ht), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), reticulocyte and nucleated RBC (NRBC) count, reticulocyte production index (RPI)] occurring in normal and PEc pregnancies and their relationship with the erythrocyte changes of their neonates. As markers of oxidative and proteolytic stress, membrane bound haemoglobin (MBH) and the profile of erythrocyte membrane protein band 3 [% of monomers, High molecular weight aggregates (HMWAg) and proteolytic fragments] was evaluated, as well as bilirubin concentration, as a marker of hemoglobin turnover. RBCs under oxidative and/or proteolytic stress are known to be marked for death by a rise in MBH and band 3 modifications.

PEc mothers presented significantly higher values for MBH, HMWAg, RBC count, Hb, Ht, reticulocyte count, RPI and bilirubin concentration; no morphological RBC changes were observed.

When comparing newborns from normal and PEc mothers, we observed similar values for HMWAg, RBC count, Hb and Ht, though significantly higher MBH, MCH, MCV, reticulocyte, RPI and NRBC values were observed, and a trend to higher values of bilirubin concentration.

Our data suggest that maternal blood changes and the abnormal remodelling of placental spiral arteries in PE, seem account for a higher RBC damage/aging/removal in both mother and fetus and may somehow compromise the placenta transfer mechanisms and fetal growth.

INTRODUCTION

Normal pregnancy leads to an inflammatory process, which seems to be enhanced in preeclampsia (PE). The development of PE is linked to a failure of trophoblastic invasion of the spiral arteries. Within these arteries, the accumulation of fibrinoid material and "foam" cells may lead to a reduced blood flow, favouring the interaction between the surrounding cells. A longer exposure of red blood cells (RBC) to oxygen metabolites and proteases produced by inflammatory cells may account for RBC damage.

We aimed to study if a continuous enhanced exposure to inflammatory activation products throughout a preeclamptic (PEc) gestation, would account for a higher RBC damage, which may compromise oxygen maternal-fetal exchange and, therefore, placental homeostasis and fetus development. We evaluated maternal erythrocyte changes [RBC count, hemoglobin (Hb), hematocrit (Ht), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), reticulocyte and nucleated RBC (NRBC) count, reticulocyte production index (RPI)] occurring in normal and PEc pregnancies and their relationship with the erythrocyte changes of their neonates. As markers of oxidative and proteolytic stress, RBC membrane bound hemoglobin (MBH) and the profile of erythrocyte membrane protein band 3 [% of monomers, high molecular weight aggregates (HMWAg) and proteolytic fragments] were evaluated, as well as bilirubin concentration, as a marker of hemoglobin turnover. RBCs under oxidative and/or proteolytic stress are known to be marked for death by a rise in MBH and band 3 modifications.

MATERIALS AND METHODS

Approval for the study was given by the Ethics Committee of the Hospital S. João, Porto. PE was defined according to established criteria as a systolic/diastolic blood pressure of at least 140/90 mmHg (after 20 weeks gestation) and proteinuria of at least 1+ (30mg/dl) on dipstick testing, both on 2 occasions, 4 to 6 hours apart[1].

The study was performed in 42 healthy pregnant women and 44 PEc pregnant women, and in their neonates. Blood was collected from pregnant women before delivery, and UCB was obtained after delivery of the placenta.

Hematologic study

RBC count, Hb, Ht, MCV, MCH, MCHC were measured by an automatic blood cell counter (Abx Micros 60). NRBC count was evaluated in Wright stained blood films. Blood films using supravital new methylene blue stain (reticulocyte stain; Sigma, St Louis, MO, USA) were prepared to evaluate reticulocyte count.

Bilirubin

Total bilirubin was quantified by a colorimetric method (Bilirubin, Randox).

Erythrocyte damage

Band 3 profile:

RBC were isolated by centrifugation in a density gradient. Washed RBC were lysed and washed according to Dodge method[2] and using PMSF (as a protease inhibitor) in the first two washes. Protein concentration of the obtained membrane suspensions was evaluated by the Bradford method[3].

After SDS-PAGE a RBC membrane proteins and electrophoretic transfer to nitrocelulose sheet, the immunoblot for band 3 was performed[4] and band 3 profile quantified by densitometry (*Bio1D++ version 99*, Vilber Lourmat, France).

Membrane bound hemoglobin (MBH):

Measured spectrophotometrically at 415 and 700 nm, expressed in function of protein content[4].

STATISTICS

Data normally distributed are presented as mean ± standard deviation and compared using the Student *t* test. Data not normally distributed are presented as medians and interquartile range (IQR) and compared using Mann-Whitney U test and Wilcoxon Signed Ranks Test. Correlations between variables were evaluated by the Spearman´s correlation coefficient (*r*). Significance was accepted at *P* less than 0.05.

RESULTS

Clinical characteristics of the studied groups are presented in Table I.

PEc pregnancy, when compared with normal pregnancy, presented significantly higher blood pressure; similar maternal age and body mass index (BMI) and significantly lower gestational age and newborn weight.

Figure 1 presents the immunoblots for band 3 of two studied cases (a normal and a PEc case).

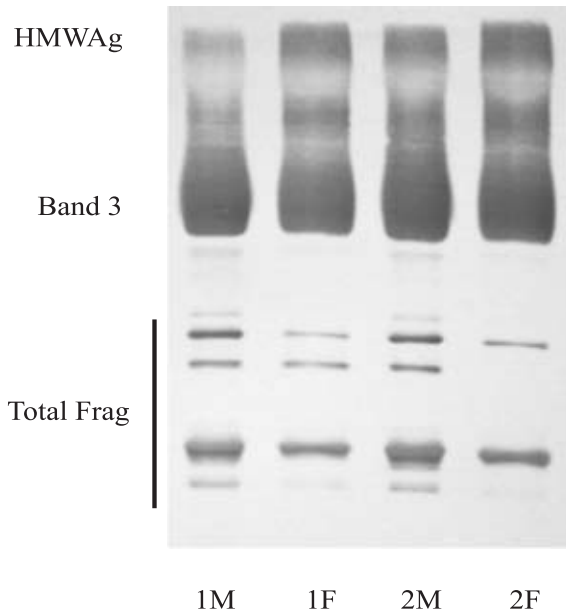

Figure 1 – Band 3 immunoblots of two studied cases. 1M: normal pregnant woman; 1F: UCB from normal pregnancy; 2M: PEc pregnant woman; 2F: UCB from PEc pregnancy.

Table I – Clinical data of normal and preeclamptic groups at delivery [mean ± SD or median (IQR)].

	Normal	Preeclamptic	P
Maternal characteristics:			
Gestational Age (wk)	38.5 (38.0; 39.3)	37.0 (34.3;38.0)	< 0.001
BMI (kg/m^2)	29.2 (27.2; 30.8)	29.8 (26.8; 33.0)	0.15
Age (y)	30.4 ± 5.7	29.7 ± 5.2	0.61
Blood Pressure (mm Hg):			
Systolic	119.9 ± 11.5	155.0 ± 14.9	< 0.001
Diastolic	69.0 ± 7.2	97.4 ± 6.3	< 0.001
Fetal characteristics:			
Birth weight (kg)	3.4 (3.0; 3.7)	2.6 (1.9; 3.1)	< 0.001
SGA (n)	0 (0%)	6 (13.6%)	< 0.01

BMI – Body Mass Index; **SGA** – Small for Gestational Age

The Table II presented the hematologic study, bilirubin levels and the band 3 profiles for the studied groups (percentage of band 3 monomer, high molecular weight aggregates; total proteolytic fragments).

Comparing normal with PEc pregnant women, we observed significantly higher RBC, Hb, Ht, reticulocyte count, RPI, bilirubin, HMWAg and MBH for PEc group.

We found that MCV, MCH, NRBC, reticulocyte count, RPI and MBH were significantly higher in UCB from PEc pregnancy, when compared with normal pregnancy.

All parameters, except MCHC and Total Frag, were significantly higher in UCB (normal and PEc) than in maternal blood.

We also found significant positive correlations between maternal and cord blood for MBH (in normal and PEc pregnancy, Fig. 2), for HMWAg (in normal and PEc pregnancy, Fig. 3) and for Bilirubin (in PEc pregnancy, Fig. 4). We also found a significant positive correlation between

Table II – Hematologic study and bilirubin levels of normal and preeclamptic groups [median (IQR)].

	Maternal		P Value	Fetal		P Value
	Normal (n =42)	PEc (n =44)		Normal (n =40)	PEc (n =44)	
RBC ($x10^{12}$/l)	3.7 (3.5; 4.0)	4.2 (3.9; 4.4)	<0.001	4.4 (4.1; 4.6)	4.3 (4.0; 4.7)	0.38
Hb (g/dl)	11.5 (10.9; 12.4)	12.7 (11.8; 13.8)	<0.001	15.5 (14.8; 16.2)	16.0 (14.7; 17.2)	0.12
Ht (%)	34.0 (32.0; 36.1)	38.5 (35.1; 40.8)	<0.001	45.9 (43.2; 49.6)	47.2 (44.5; 50.8)	0.28
MCV (fl)	91.0 (87.8; 94.0)	91.6 (89.1; 94.4)	0.18	104.5 (103.0; 107.8)	110.0 (106.9; 114.6)	<0.001
MCH (pg)	30.6 (29.8; 31.9)	30.8 (29.8; 31.9)	0.73	35.5 (34.3; 36.2)	36.5 (35.6; 38.5)	<0.001
MCHC (g/dl)	33.9 (33.1; 34.6)	33.5 (32.9; 34.2)	0.15	33.6 (33.0; 34.2)	33.7 (32.9; 34.3)	0.51
Reticulocytes ($x10^{9}$/l)	36.0 (22.8; 57.8)	71.4 (47.3; 96.3)	<0.001	154.0 (96.6;191.2)	158.0 (138.1;205.3)	0.048
RPI	0.60 (0.30; 0.84)	1.40 (0.90;1.90)	<0.001	3.40 (2.20; 4.60)	4.00 (3.20; 5.20)	0.020
NRBC ($x10^{9}$/l)				0.39 (0.16; 0.90)	0.59 (0.28; 1.69)	0.016
Bilirubin (mg/dl)	0.50 (0.40; 0.70)	0.80 (0.60; 0.80)	0.001	1.30 (1.13; 1.56)	1.50 (1.13;1.78)	0.086
MBH (%$x10^{-4}$)	73.0 (66.3; 95.8)	86.0 (70.5; 126.0)	0.016	228.8 (188.3; 294.3)	331.0 (245.5; 420.3)	<0.001
HMWAg (%)	14.9 (10.7; 18.3)	16.3 (13.7; 18.9)	0.040	19.9 (17.7; 22.8)	19.5 (18.5; 21.8)	0.61
Band 3 (%)	57.1 (51.9; 62.0)	54.0 (49.0; 60.9)	0.17	60.7 (56.0; 65.4)	61.2 (55.4; 65.5)	0.77
Total Frag (%)	28.0 (21.9; 35.3)	29.4 (20.9; 34.9)	0.80	17.3 (14.1; 25.8)	18.0 (14.4; 25.3)	1.00

Total frag, includes proteolytic fragments (60, 40 and 20 kDa); PEc, Preeclamptic
(a) Mother/Fetus in normal pregnancy; (b) Mother/Fetus in PEc pregnancy

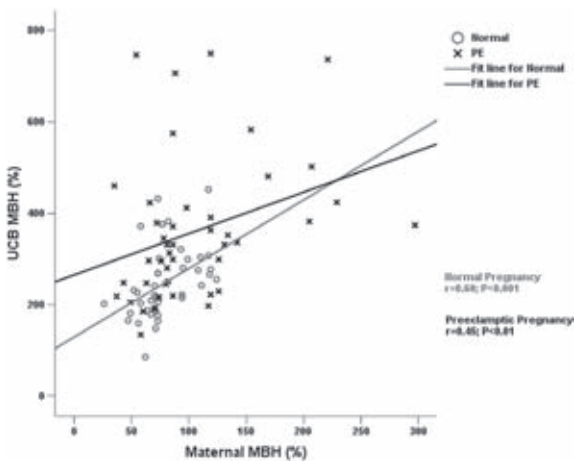

Figure 2 – Maternal vs cord blood MB

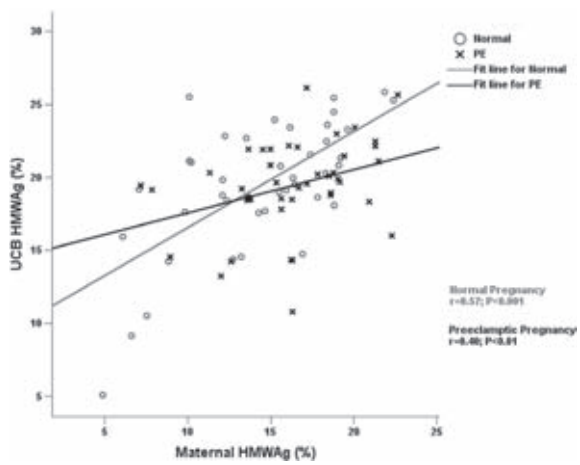

Figure 3 – Maternal vs cord blood HMWAg

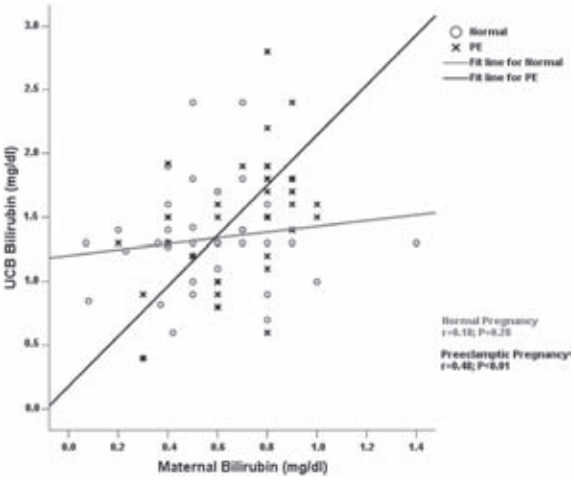

Figure 4 – Maternal vs cord blood bilirubin

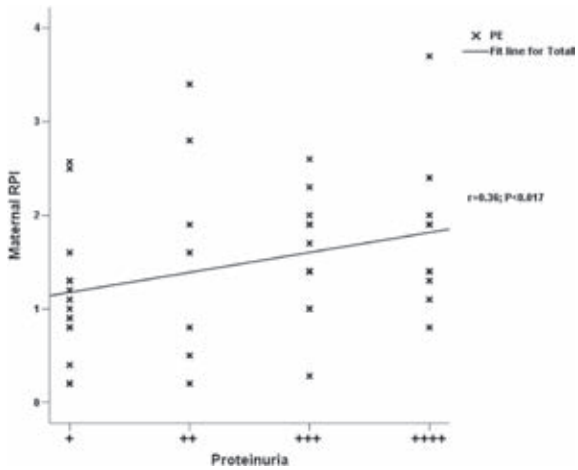

Figure 5 – Proteinuria vs Maternal RPI

maternal RPI and Proteinuria (marker of PE severity) (Fig. 5).

CONCLUSIONS

Our data show a higher RBC damage in both mother and fetus in PE that might be linked to maternal blood changes and to hemorheological disturbances due to the abnormal remodelling of placental spiral arteries[5]. Our data suggest that in PEc women a higher RBC damage/removal occurs, as suggested by the significant increase in bilirubin, in MBH and in band 3 aggregation (HMWAg), triggering a higher physiological RBC production, as reflected by the higher reticulocyte count and RPI values. This increase in RBC damage and/or removal in PEc mothers and in their newborns may be also a reflection of a placental hypoxic condition. A relationship between maternal and fetal changes seems to occur, as suggested by the observed correlations between maternal and fetal markers of RBC damage. Moreover, we propose MBH and band 3 profile as good markers of RBC damage in PE.

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