

ALTERED INTRAHEPATIC HEMATOPOIESIS IN NEONATES FROM WOMEN WITH PREGNANCY INDUCED HYPERTENSION/PRE-ECLAMPSIA

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Summary: Aim: To detect whether preeclampsia influences neonatal intrahepatic hematopoiesis, given that an activation of fetal neutrophils and monocytes during the course of this disorder occurs. Experimental design: We examined liver samples from 10 neonates of hypertensive/preeclamptic women at 27 to 28 weeks of gestation delivered by a cesarean section. All neonates were placed in incubators but they all died within 24 hours due to immaturity. The control group comprised 10 fetuses of the same gestational age, after voluntary abortion due to a neural defect. Specific antibodies against CD34, glycophorin C, hemoglobins A and F, myeloperoxidase, CD61, CD68, terminal deoxynucleotidyl transferase and the pax-5/B-cell specific activator protein, were used in each sample. Results: Neonates from hypertensive/preeclamptic women, in comparison with controls, showed: a statistically significant reduction of erythropoiesis by 25% ($p=0.015$); a statistically significant increase of granulopoiesis ($p=0.019$); a statistically significant increase in the expression of CD68 positive cells of the monocytic lineage ($p=0.017$); a statistically significant increase in the expression of CD34 progenitor/stem positive cells ($p=0.021$). No statistically significant differences were observed in both examined groups, concerning megakaryopoiesis and B lymphopoiesis. Conclusions: Preeclampsia of pregnancy has an impact on neonatal intrahepatic hematopoiesis by increasing granulopoiesis, reducing erythropoiesis and triggering endothelial and stem cell activation. We suggest that these findings reflect a state of persistent inflammation and a loss of red blood cell production possibly contributing to the neonatal morbidity related to this disorder.

Key words: Preeclampsia; Neonatal liver hematopoiesis; Hematopoietic markers

Introduction

Preeclampsia, a severe hypertensive disorder in pregnancy, represents one of the major causes of maternal and fetal mortality in developing countries. The etiology of the condition is unknown, but placental disorders are probably involved in the pathophysiologic mechanism (16). Both the fetus and the placenta are often growth retarded when hypertension occurs before mid-gestation. There are ischemic changes and often-placental infarcts. Onset of preeclampsia in early gestation greatly increases the risk of spontaneous abortion, abruptio placenta, fetal death, spontaneous pre-term labor, and pre-term delivery.

In mid-trimester fetuses the principal site of hematopoiesis is the liver, where two functionally different compartments can be observed. Erythropoiesis mainly takes place within the sinusoids of the parenchyma while granulopoiesis is restricted to the mesenchymal tissue of the portal fields.

Impaired intrahepatic hematopoiesis has been demonstrated mainly in fetuses with chromosomal abnormalities such as Trisomy 21 (Down's syndrome) (1, 8, 23) and Trisomy 13 (10, 18, 27), and lesser in fetuses with viral infections (7, 13, 6, 14, 12).

It is known that activation of fetal neutrophils and monocytes during preeclampsia involves enhanced chemokine activation, which contributes to the fetal morbidity of this disorder (17). To elucidate whether this activation of fetal neutrophils and monocytes is associated with respective changes at the level of intrahepatic hematopoiesis, we examined by using immunohistochemistry liver samples from neonates from preeclamptic women and correlated our findings with those after voluntary abortion (control group)

Materials and methods

Ten newborns with gestational age 27 to 28 weeks, from hypertensive/preeclamptic women delivered by a cesarean

section, were examined. All subjects were admitted to the intensive care unit for neonates. Incubators were used to maintain an environment with controlled temperature, humidity and oxygen for development of the premature infants. However, none of them could make it within the next 24 hours due to immaturity. All cases fulfilled the criteria of severe preeclampsia which were based on the definitions given by Davey and MacGillivray (4). Gestational hypertension was defined as the occurrence, in a previously normotensive woman, of a diastolic blood pressure 90 mmHg on at least two consecutive occasions after the 20th week of gestation. Preeclampsia was defined as the association of gestational hypertension with significant proteinuria (>300 mg/l in a 24-h urinary collection). The corresponding placentas showed increased amounts of perivillous fibrin, dense leukocytic infiltration, and more than 15% of the parenchyma was affected by infarction. The histologic examination of the placenta was done according to a standardized protocol stating signs of developmental disturbance and with particular reference to indices of infection.

An equal number of fetuses at the same gestational age after voluntary abortion due to a neural tube defect, were used as controls. No microscopic placental changes have been found. The study was carried out in harmony with the guidelines for the analysis of fetal cells and tissues (26) and approved by the Regional Committees of Ethics. Written informed consent was obtained from all women, and the procedures followed were in accordance with the institutional guidelines.

Tissue sections from both lobes of the liver for each case were prepared. For conventional analysis the sections were stained with hematoxylin and eosin (H&E). To detect a variety of antigens related to hemato- and lymphopoiesis, a panel of monoclonal and polyclonal antibodies was applied. The antibodies used were directed against CD34 (clone QBEnd 10), glycophorin C (clone Ret40), hemoglobins A and F (polyclonal), myeloperoxidase (MPO-7, polyclonal), CD61 (clone Y2/51), CD68 (clone KP1 and PG-M1), terminal deoxynucleotidyl transferase (TdT, polyclonal) and the pax-5/B-cell specific activator protein (clone 24). With the exception of the anti-CD34 and anti-pax-5 antibodies, which were obtained from Coulter-Immunotech, Krefeld, Germany, and Becton Dickinson Biosciences, Lexington, Kentucky, U.S.A., respectively, all other antibodies were purchased from DAKO, Glostrup, Denmark. For demasking of most of the antigens a high pressure cooking technique was used applying 10mM citrate buffer pH 6.0 while demasking glycophorin C and CD61 required proteolytic treatment. Bound antibodies were visualized employing the alkaline phosphatase (APAAP) method and Fast Red for development. For identification of mature granulopoietic cells, the histochemical detection of Naphtol-AS-D-chloroacetate esterase (SIGMA Diagnostics, St Louis, MO, USA) was employed.

The statistical analysis was obtained using the non-parametric Mann-Whitney test. The mean values were expressed as average SD.

Results

Five microscopic fields of the parenchyma of the liver were evaluated in each case without knowledge of the clinical data, and the number of stained cells per square millimeter was calculated. We identified islands of hematopoietic cells using conventional histology within the fetal liver parenchyma. Immunohistochemical analysis then facilitated the precise definition of the various lineages. The sections were examined independently by two observers, and positive cellular staining for each antibody was manifested as fine red cytoplasmic granularity.

The liver of fetuses after voluntary abortion showed a distinct distribution of hematopoiesis. Erythropoiesis (evidenced by positive expression of glycophorin C, and hemoglobins A and F) occurred in aggregates mainly within the sinusoids of the hepatic parenchyma (averaged of 3820640 cells/mm²; range 2800–4900 cells/mm²) (Fig. 1). Granulopoiesis (based on the detection of positive expression of myeloperoxidase and chloroacetate esterase) occurred primarily within the mesenchymal stromal cells of the portal fields (averaged: 32.517.8 cells/mm²; range 12–75 cells/mm²) (Fig. 2). Monocytopoiesis (documented by positive expression of CD68) was found within the mesenchymal stromal cells of the portal fields (averaged: 2.650.73 cells/mm²; range 1.8–3.9 cells/mm²) (Fig. 3). The anti-CD34 antibody led to labeling of the endothelial cells of numerous sinuses, while numerous CD34-positive blasts mainly within the stroma of the portal fields, were discernible (averaged: 45.715.2 cells/mm²; range 18–73 cells/mm²). Furthermore, a number of cells expressing CD61 were identified; this is characteristic for megakaryocytes. Using morphological criteria, these cells comprised a spectrum ranging from small cells with unilobated nuclei to large multilobated megakaryocytes (averaged: 3.120.38 cells/mm²; range 2.1–4.2 cells/mm²). The antibody specific for pax-5/B-cell specific activator protein labeled single nucleated cells corresponding to B lymphocytes (averaged: 5.680.96 cells/mm²; range 4.2–7.8 cells/mm²). Terminal deoxynucleotidyl transferase (TdT)-positive cells were not identified either in the portal fields, or in any other region of the fetal hepatic sample.

Morphological and immunohistological analysis of the neonatal liver samples from hypertensive/preeclamptic women showed: 1) A severe reduction of erythroid cells by 25% (averaged: 2890760 cells/mm²; range 2300–4500 cells/mm²) (Fig. 4) which was statistically significant ($p=0.015$). 2) An intense granulopoietic activity in the mesenchymal tissue of the portal fields extending into the sinusoids (Fig. 5). The number of granulopoietic cells was more than 2 times higher than that found in the voluntary abortion group (averaged: 70.432.7 cells/mm²; range 25–160 cells/mm²). Comparing the granulopoietic activity in the two groups we found a statistically significant difference ($p=0.019$), favoring the preeclamptic pregnancies. A similar pattern was observed for the monocytic lineage (averaged:

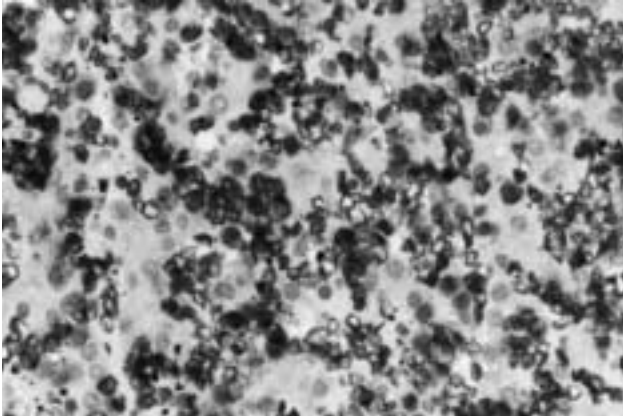


Fig. 1: Control group: Erythropoietic cells within the hepatic sinuses expressing glycophorin C. Immunostaining using APAAP technique (red labeled cells), X400.

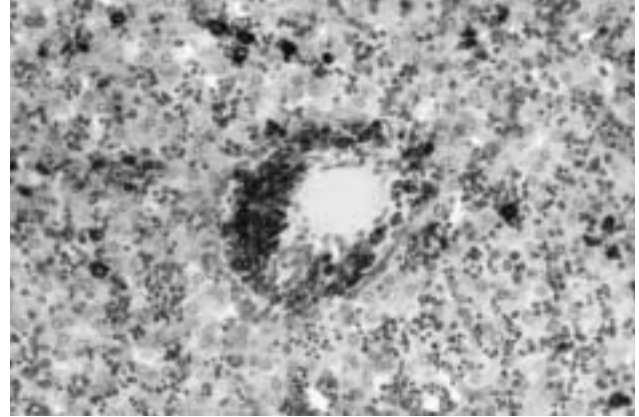


Fig. 2: Control group: Myeloperoxidase-positive cells of granulocytic lineage within the mesenchymal tissue of the hepatic portal fields. Immunostaining using APAAP technique (red labeled cells), X200.

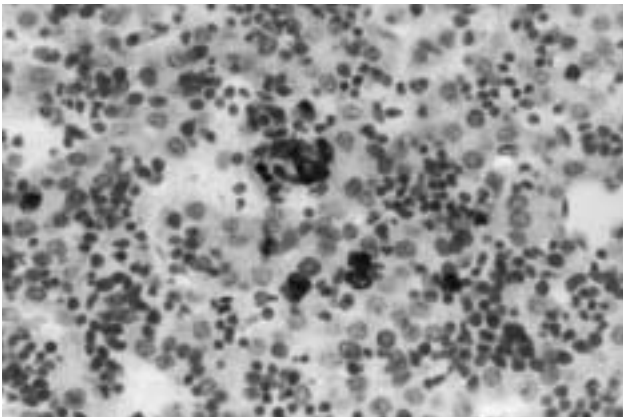


Fig. 3: Control group: Monocytes expressing the CD68 antigen. Immunostaining using APAAP technique (red labeled cells), X400.

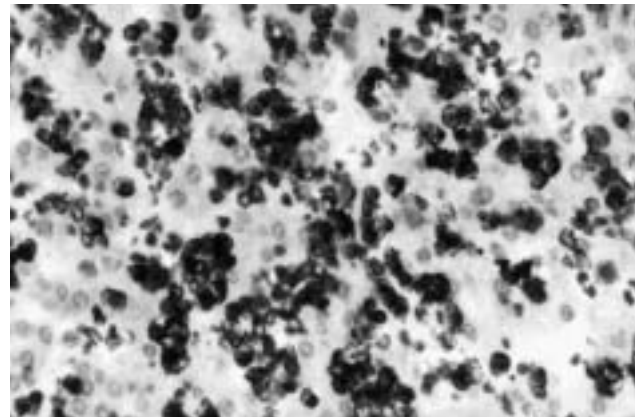


Fig. 4: Preeclamptic group: Erythropoietic cells within the hepatic sinuses expressing glycophorin C. Immunostaining using APAAP technique (red labeled cells), X400.

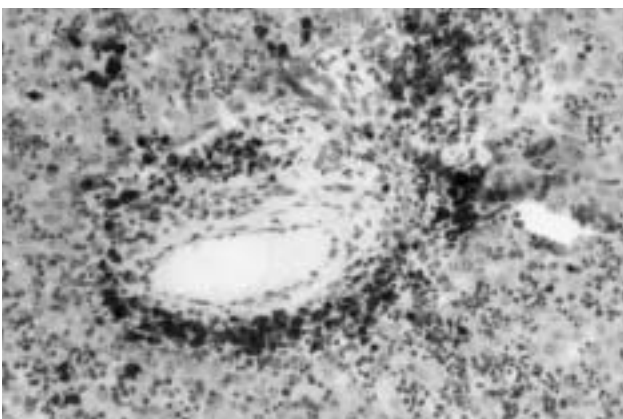


Fig. 5: Preeclamptic group: Myeloperoxidase-positive cells of granulocytic lineage within the mesenchymal tissue of the hepatic portal fields. Immunostaining using APAAP technique (red labeled cells), X200.

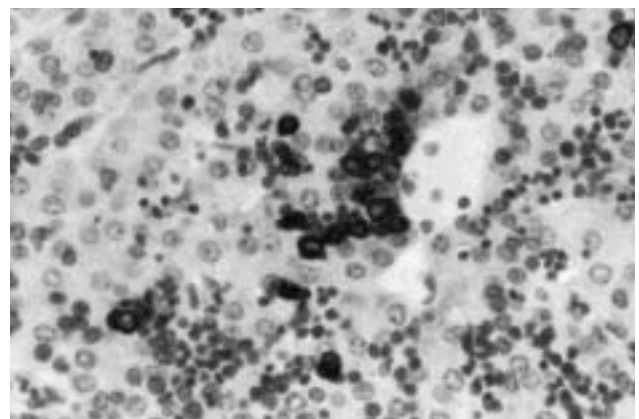


Fig. 6: Preeclamptic group: Monocytes expressing the CD68 antigen. Immunostaining using APAAP technique (red labeled cells), X400.

Tab. 1: Descriptive characteristics of the liver samples from the two examined groups (27th –28th weeks of gestation).

	Control group	Preeclamptic group	Statistical analysis
Erythropoiesis (cells/mm ²)	3820±640	2890±760	p=0.015
Granulopoiesis (cells/mm ²)	32.5±17.8	70.4±32.7	p=0.019
Monocytopoiesis (cells/mm ²)	2.65±0.73	3.65±0.81	p=0.017
Progenitor hematopoietic cells and vascular endothelial cells (cells/mm ²)	45.7±15.2	68.1±20.8	p=0.021
Megakaryocytopoiesis (cells/mm ²)	3.12±0.38	3.21±0.79	NS
B Lymphopoiesis (cells/mm ²)	5.68±0.96	5.85±1.08	NS

3.650.81 cells/mm²; range 2.3–4.8 cells/mm²) (Fig. 6). Comparing the two examined groups at the level of CD68 we found a statistically significant difference (p=0.017) favoring the preeclamptic deliveries. The quantitative expression of CD34 by the vascular endothelial cells, the progenitor/stem hematopoietic cells and stromal progenitor cells was more intense in the mesenchymal portal tissue and the extending sinusoids (averaged: 68.120.8 cells/mm²; range 32–93 cells/mm²). Comparing the two examined groups at the level of CD34 we also found a statistically significant difference (p=0.021) favoring the preeclamptic cases. No significant quantitative differences concerning megakaryopoiesis and B lymphopoiesis, were observed, between the two groups. The above results are summarized in table 1.

Discussion

Pre-eclampsia of pregnancy refers to a symptom complex characterized by hypertension, proteinuria and edema. It occurs in about 7% of primiparous women, usually after 20 weeks of gestation (4). Endothelial dysfunction appears to be central in the pathophysiology of this disorder (19) and has also been reported in the fetus (5, 28) but the mechanisms leading to this dysfunction have not been clarified. In preeclampsia, there is a faulty shallow placentation, i.e., a reduced invasion of the trophoblast into the uterus and its spiral arteries (3), resulting in a significant reduction in the uteroplacental blood flow (15), with a chronic prenatal fetal hypoxia. Hypoxia has been shown (1) indirectly, in the human umbilical artery and vein, where abnormal Doppler velocity waveforms indicate an increased vascular resistance (25) and hypoxia (11); (2) directly, by increased plasma adenosine (29) and erythropoietin levels (20) reduced pH and PO₂ levels, and increased PCO₂ levels in the human umbilical artery and human umbilical vein (21, 24); and (3) clinically, by increased frequency of placental infarcts (2). Long-range uteroplacental hypoxemia may produce inflammatory changes in the placenta with the release of inflammatory chemokines, inducing fetal neutrophils' and monocytes' activation, which, in turn, may release additional inflammatory mediators (22). This may make a vicious circle, leading to strong CD34 positive cell activation.

To give insight into possible mechanisms of neonatal morbidity in preeclampsia we focused on intrahepatic he-

matopoiesis, in comparison to controls after voluntary abortion. In our settings, the comparative study of the quantitative percentage of:

- Glycophorin C and hemoglobins A and F, showed a reduction of erythropoiesis in preeclamptic pregnancies which was statistically significant (p=0.015)
- myeloperoxidase and chloroacetate esterase showed a statistically significant elevation of granulopoiesis in preeclamptic deliveries (p=0.019)
- CD68 for the identification of monocytes showed a statistically significant elevation of monocytopoiesis in preeclamptic deliveries (p=0.017)
- CD34 for the identification of progenitor hematopoietic cells and vascular endothelial cells showed an enhanced hematopoiesis in preeclamptic fetuses over controls (p=0.021)

The quantitative percentages of CD61 and pax-5/B-cell specific activator protein, showed no differences concerning megakaryocytopoiesis and B-lymphopoiesis between preeclamptic and control groups.

Our data imply a loss in red blood cell production and a persistent inflammatory response in preeclampsia, which could explain the morbidity of this disorder. Further investigation is necessary to establish this hypothesis.

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