Nucleated Red Blood Cell Count in Maternal Peripheral Blood and Hypertensive Disorders in Pregnant Women

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ABSTRACT

Objective: We investigated the correlations of nucleated red blood cell (NRBC) counts with hypertensive disorders in pregnancy (HDP) and fetal umbilical blood flow velocity.

Materials and Methods: We recruited 282 patients with HDP as experimental group including 107 with mild pre-eclampsia (A1 group), 100 with severe pre-eclampsia (A2 group) and 75 with eclampsia (A3 group), and 215 normal pregnant women as control group. Maternal peripheral venous blood was collected and isolated cells were stained with Wright-Giemsa. We estimated NRBC counts according to laboratory routine and Doppler ultrasound examinations were employed to measure the systolic/diastolic (S/D) ratios of fetal ductus venosus, umbilical artery and middle cerebral artery.

Results: The NRBC counts in A1, A2 and A3 groups were higher than control group (all $P < 0.01$). The S/D ratios in control, A1, A2 and A3 groups increased orderly ($P < 0.05$). Receiver operating characteristic curve analysis demonstrated that the sensitivity and specificity of NRBC count and S/D ratios in diagnosing HDP were 96.50% and 96.28%; 93.60% and 98.14%; 94.30% and 94.88% 99.30% and 100%, respectively. Pearson and Spearman correlation analysis revealed that the NRBC and S/D ratios were correlated with gestational age at birth, amniotic fluid volume, premature birth, mechanical ventilation, neonatal intensive care unit admission, neonatal asphyxia, birth weight, fetal distress, APGAR score, pH value, arterial oxygenation tension, bicarbonate and base excess (all $P < 0.05$). The NRBC count was positively associated with the S/D ratios (all $P < 0.05$).

Conclusions: Our results provide evidence that NRBC count in patients with HDP increased significantly, showing positive correlations with umbilical S/D ratios.

Key Indexing Terms: Hypertensive disorders in pregnancy; Nucleated red blood cells; Umbilical S/D ratio; Pearson's correlation test; Fetus survival. **[Am J Med Sci 2016;351(2):140–146.]**

INTRODUCTION

Hypertensive disorders in pregnancy (HDP) is the most frequent health problem occurring during pregnancy and the leading cause of high perinatal, infant and fetal morbidity and mortality.[1,2] HDP is defined clinically as chronic hypertension before pregnancy or before 20 weeks of gestation, gestational or pregnancy-induced hypertension after 20 weeks of gestation and pre-eclampsia (PE) and eclampsia, which progresses to tonic-clonic seizures.[3] HDP is life-threatening for both the mother and the child and affects approximately 10-16% of pregnant women globally.[4] The risk factors of HDP include the family history of PE, advanced maternal age, first pregnancy, pre-existing hypertension, diabetes mellitus, obesity and maternal smoking.[5,6] Typically, the clinical presentation of HDP involves hypertension, proteinuria and edema.[7] It has been observed in previous studies that the measurement of maternal plasma inhibin A, combined with uterine artery Doppler measurements in the first trimester or early second trimester, is an effective screening method to monitor the progression of HDP and PE.[8,9]

Nucleated red blood cells (NRBC) that are immature red blood cells, are commonly detected in the umbilical and peripheral blood of newborns and are indicators of hematopoiesis in neonates.[10] Maternal NRBC counts can predict perinatal complications as it is closely correlated with intraventricular hemorrhage, bronchopulmonary dysplasia, necrotizing enterocolitis and death.[11-13] Fetal hemorrhage has also been associated with increased maternal NRBC count, and elevated maternal NRBC count at birth is attributed to fetal hemorrhage, intrauterine growth retardation or fetal stress in the fetus of mothers with HDP.[14-16] The increased fetal NRBC count in premature infants delivered from patients with PE is the primary marker for detection of adverse fetal outcomes.[17] Hypoxia induces fetal compensatory reactions to increase hematopoiesis as well as the inflow of premature red blood cells into the neonatal blood flow, indicating prenatal hypoxia.[18] We investigated whether there are correlations between NRBC counts and the severity of HDP, as well as between NRBC counts and the changes in fetal umbilical blood flow velocity, and explored whether the NRBC count could be a rationale

for diagnostic applications in the prenatal prediction of HDP.

MATERIALS AND METHODS

Ethics Statement

The study received approval from the Ethics Committee of Division of Birth Cohort Study, the First Affiliated Hospital of the Second Military Medical University. All subjects have provided written informed consent before the study. Study protocols were based on the ethical principles for medical research involving human subjects of the Helsinki Declaration.[19]

Study Subjects

Between January 2007 and January 2014, a total of 282 patients (mean age, 27.15 ± 3.10 years; mean gestational age, 27.80 ± 4.51 weeks) diagnosed with HDP were selected as the experimental group. Among these patients, 107 patients were diagnosed with mild PE (A1 group), 100 patients with severe PE (A2 group) and 75 patients with eclampsia (A3 group), according to the criteria used for the clinical diagnosis of HDP referred to as the Australasian Society for the Study of Hypertension in Pregnancy criteria.[20] In addition, 215 normal pregnant women (mean age, 27.00 ± 2.42 years; mean gestational age, 27.19 ± 4.30 weeks) were randomly selected as the control group. The control group was normal for all the rigorous examinations conducted, including blood pressure, urine routine, liver and kidney function, electrolytes, electrocardiogram and Type-B sonic to exclude the organic disease.

Sample Collection

Maternal peripheral venous blood samples (6 mL) were collected from the experimental and control groups, and placed in ethylenediaminetetraacetic acid tubes at room temperature, which were used for analysis within 6 hours. The samples were diluted at 1:1 ratio with 0.9% normal saline and layered in 2 sterile tubes containing 3 mL lymphocyte separation medium. After centrifugation at 2000 rpm for 30 minutes, the interphase layer cells were carefully removed and washed with 0.9% normal saline 2 times before preparing smears.

NRBC Counts

Venous blood samples for complete blood cell counts were collected from each pregnant woman. The smears were stained by Wright-Giemsa (Beijing Midwest Technology Co, Ltd) for 10 minutes. Then, the NRBC counts were analyzed according to a laboratory routine using a high-power microscope (Olympus CH20-BM, Japan). NRBCs with the following histologic features: round or oval cells with a diameter of 15-18 μm after Wright-Giemsa staining; round nucleus with deep purple chromatin with nucleolus absent; large cytoplasmic volume and stained into bluish purple or blue with obvious lightly staining perinuclear cytoplasm and the ratio of nucleus and cytoplasm 1:2, were defined as normoblasts.

Doppler Ultrasound Examination

The height (cm), weight (kg) and estimated gestational age of all pregnant subjects were measured before Doppler ultrasound examination. The Doppler ultrasound examination of the fetal ductus venosus (DV), umbilical artery and middle cerebral artery was performed within 1 week before delivery using a Philips I-U22 ultrasonic color Doppler diagnostic apparatus (Philips Healthcare, Bothell, WA) with a 3.5 MHz transabdominal transducer. All the scans were performed by an experienced observer and the ultrasound data were prospectively entered into a computer database. The pregnant women were placed in a supine position in a quiet state. The Color Doppler was used to identify fetal growth, fetal heart rate, biparietal diameter, placenta, amniotic fluid volume, resistance index, pulsatility index as well as the ratio of peak systolic velocity/peak diastolic velocity (systolic/diastolic [S/D]) of the fetal DV, umbilical artery and middle cerebral artery. The fetal DV was measured at mid-sagittal or oblique section of the fetal upper abdomen, with a probe moving along the fetal head laterally until the ultrasonographic long-axis view of the umbilical vein was seen clearly. Measurements were taken at this point before the umbilical vein branched into the left branch of the portal vein, and an exiguous tubular structure was probed into connecting with the postcava. The DV had obvious blood flow signals in the Color Doppler ultrasound imaging. Measurements were taken in the distal end of the DV with a sampling volume inside the blood vessels. As for the measurement of umbilical artery, an ultrasound-guided sampling of the umbilical cord was performed between both ends of the umbilical artery with a sampling volume of 2-4 mm. The Doppler was used to show the blood flow spectrum. The measurements of middle cerebral artery were taken at the center of the umbilical venous lumen with a sampling volume of 2-4 mm, and with an angle of insonation of less than 30°. Angle correction was then applied, and the spectral Doppler signals from 3 similar consecutive waveforms were obtained. When continuous and stable Doppler flow velocity waveforms of the fetal DV, umbilical artery and middle cerebral artery were obtained, the freezing measure was performed.

Statistical Analysis

We used the statistic software SPSS19.0 (SPSS Inc, Chicago, IL) to carry out statistical analysis. The comparison of enumeration data between groups was performed by using the I^2 test. Measurement data were presented as mean ± standard deviation ($x \pm s$), and examined by the test of normality. The comparison of measurement data between the 2 groups was performed by using the t test and among multiple groups by using