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Prediction of Preeclampsia with Elevation in Erythroblast Count in Maternal Blood.

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Abstract

Introduction: The predominant etiologic theory of preeclampsia is that reduced uteroplacental perfusion is the unique pathogenic process in the development of preeclampsia. Maternal and fetal erythroblast counts are elevated in the peripheral blood of pregnant women with preeclampsia. The purpose of this study was to examine whether this elevation actually occurs before the clinical onset of the disorder.

Study design: In a prospective cohort survey erythroblasts were enumerated in 599 maternal blood samples obtained in 19-26 weeks with singleton pregnancy. After complete blood count a peripheral blood smear was done and erythroblast was counted, and results were subsequently correlated with pregnancy outcomes. The data were analyzed by SPSS 13.0. Independent sample t-test and Fisher's exact test was used. A p value of <0.05 was considered statistically significant.

Results: Significantly higher quantities of erythroblasts (mean 2.46 ± 1.23 vs. 0.44 ± 0.55 ; $p=0.009$) were detected in blood samples obtained from women who later acquired preeclampsia ($n=50$) than in blood samples from the control Cohort ($n=549$). Intrauterine growth restriction was accompany by a similar rise in erythroblast count (mean NRBC 0.82 ± 0.8 in preeclamptic group vs. 0.59 ± 0.85 in normotensive group; $p=0.009$). Mean gestational age was less in preeclamptic group (37.58 ± 1.45 vs. 39.07 ± 0.94 , $p=0.009$). On the basis of 1.5 erythroblast as point of convergence there was sensitivity =61.45, specificity=93.02, NPV=98.16, accuracy=91.65

Conclusion: Because a large proportion of the erythroblasts in maternal blood are fetal origin, our data suggest that fetal-maternal cell traffic is affected early in pregnancies that are later complicated by preeclampsia.

Key Words: erythroblasts, preeclampsia, IUGR, fetal cell traffic.

Introduction:

Hypertensive disorders in pregnancy, especially preeclampsia, remain a major cause of maternal and infant morbidity and mortality worldwide ⁽¹⁾. It is a multisystem disorder characterized by hypertension and proteinuria that occurs late in the second or, more frequently, in the third trimester of pregnancy. The disease adversely affects 3-5% of pregnancies and is one of the most important causes of maternal and fetal mortality and morbidity in developed countries. It is associated with substantial risks, such as intrauterine growth restriction with attendant complications for the fetus, prematurity, and death. The mother becomes at risk of seizures (eclampsia), renal failure, pulmonary edema, stroke, and death. Despite numerous basic, clinical, and epidemiologic studies that have been conducted over the past half-century, knowledge of the etiology and pathogenesis of preeclampsia remains elusive ⁽²⁾. Because the pathophysiology of preeclampsia has not yet been elucidated, clinical trials have failed to demonstrate any effective prevention or treatment strategies, apart from early delivery in cases where the disorder is severe ^(3,4).

A prevailing hypothesis regarding the pathogenesis of preeclampsia is the "ischemic model." Decreased uteroplacental perfusion is hypothesized to be the primary step and the point of convergence of diverse pathogenic processes in the development of preeclampsia ^(5,6). It is intuitive that reduced placental blood flow should result in decreased fetal growth, with an increased

risk of intrauterine growth restriction and low birth weight. However, epidemiologic studies have not conclusively established an association between preeclampsia or gestational hypertension and poor fetal growth ⁽⁷⁾.

Preeclampsia and intrauterine growth restriction (IUGR) are serious complications of pregnancy associated with high mortality and morbidity. Although the precise etiology of these conditions remains unclear, there is substantial evidence that they occur because the trophoblast is unable to effectively invade the decidua or to modify the spiral artery walls ^(8,9,10). At present, there is no reliable test to identify women at risk for developing these disorders early enough in pregnancy to permit preventive treatment and treatment is therefore usually symptomatic for hypertension.

Recently, it has been demonstrated that fetal cells, as well as fetal DNA, in maternal circulation in weeks 22 and 23 of gestation precede the onset of preeclampsia, suggesting that impaired placental perfusion is associated with an increase in fetomaternal trafficking ^(11,12,13,14). Because preeclampsia is a major cause of fetal and maternal morbidity and mortality, it is important to develop a predictive screening test early in pregnancy so that we can anticipate pregnancies at high risk for this complication

It is widely accepted that fetal cells and cell-free DNA are present in the maternal circulation, so both are currently being investigated as tools for non-invasive prenatal diagnosis ^(15,16,17,18,19,20). Many researchers have selected nucleated red blood cells (NRBC) as the target cells for

the development of a non-invasive method of prenatal diagnosis in maternal blood. Erythroblasts have several advantages over other types of fetal cells, such as their relative abundance in the early fetal circulation, the expression of antigens that allows their enrichment and identification, and their short life span, which precludes the isolation of fetal cells from previous pregnancies ^(21,22,23,24,25).

Although the symptoms of preeclampsia become apparent only in the second half of pregnancy, the underlying pathological causes in the placenta occur much earlier ^(26,27). No reliable test exists to identify women at risk of developing the disorder early enough in their pregnancies to permit preventive treatment. In the last few years, however, several studies described the elevation in fetal cell traffic and the increased release of fetal DNA into maternal circulation of pregnant women affected by preeclampsia ^(28,12,13,29,32). Because the initiating lesion of preeclampsia is most likely to be placentally related, this perturbation in cell trafficking could be caused by the underlying placental dysfunction. With our observation as a basis we determined to investigate whether such an elevated cell traffic of fetal cells could be detected early during pregnancies subsequently complicated by preeclampsia.

Materials and Methods:

In a prospective cohort survey 599 pregnant women at 19-26 weeks in September 2005 to December 2006 attending the Department of Obstetrics and Gynecology at

the Hospital University of Mirza kochak khan were recruited for the study with informed consent. All pregnancies were singleton. Women were healthy, normotensive on no medication, and carried fetuses with no obvious defects and normal growth. Ethical approval for this study was provided by ethical committee of Tehran university medical sciences. Informed consent was obtained in all instances.

Exclusion criteria were history of haematologic disorders that cause increment in erythroblasts like leukemia, thalasemia or every type of anemia that was cured or is curing or injection of erythropoietin or history of bleeding during pregnancy or chronic hypertension (Blood pressure $\geq 140/90$ mm Hg before 20 weeks of gestation)

Maternal blood samples were collected for complete blood count from pregnant women. If CBC was normal ($Hb > 11$), we would take a peripheral blood smear. Slides were air-dried before being fixed in methanol and stained with May/Grünwald/ Giemsa to detect the presence of erythroblast and the number of erythroblasts was enumerated by using an axiolescope light microscope. All pregnant women followed until delivery for preeclampsia and neonatal outcome. Preeclampsia is defined as a blood pressure of 140/90 mmHg with proteinuria of 1+ on dipstick in two samples taken 6 hours apart, or >0.3 g in a 24-hour urine collection.

Birth weight was measured shortly after delivery. Gestational age was based on the last menstrual period, confirmed by early pelvic examination, and verified by first-

trimester or early second-trimester ultrasound when available. If the date of the last menstrual period was not thought to be accurate, gestational age was based solely on the first-trimester or early second-trimester ultrasound findings. Pre-term labor was defined as delivery before 37 weeks of pregnancy. Details of the pregnancy outcomes were available from the patients' files. The diagnosis of IUGR was made if the birth weight was below the fifth percentile of the normal range of gestation. The mean gestational age at birth, systolic and diastolic blood pressure, neonatal birth weight, IUGR and erythroblast count were evaluated in normotensive and preeclamptic groups. The data were analyzed by SPSS 13.0 Statistical Software Package for Windows (SPSS Inc., Chicago, IL). Independent sample t-test and Fisher's exact test was used. A p value of <0.05 was considered statistically significant.

Results:

Fifty of the 599 women developed preeclampsia. The mean age of pregnant women was 26.22 ± 3.4 years old (Min=19, Max=37). Demographic data are showed in table 1.

The most frequent group was 26-30 years old (48.08%, 217 women). Mean gestational age at birth was 37.58 ± 1.45 weeks in preeclamptic groups versus 39.07 ± 0.94 weeks in normotensive groups. Birth weight at delivery was 2796 ± 399 gr versus 3248 ± 312 gr respectively. We also com-

pared the number of NRBC in pregnancies with preeclampsia versus the normotensive group. The median of erythroblasts was higher in the group with preeclampsia than in the group of normotensive pregnant women (2.64 ± 1.23 versus 0.44 ± 0.55 , NRBC respectively), the difference was statistically significant $p=0.009$. We found significantly higher ratios of NRBC in samples from IUGR neonates 0.82 ± 0.8 . In the appropriate growth neonate, isolated NRBC was 0.59 ± 0.85 , the difference was statistically significant ($p=0.044$). NRBC in preterm labor group was 2.59 ± 1.66 versus 0.55 ± 0.74 in term labor group. NRBC in IUGR and preeclampsia group was 1.9 ± 0.87 versus 0.6 ± 0.57 in IUGR without preeclampsia. The difference was significant ($p=0.001$). NRBC in preeclampsia and preterm labor group was 3.23 ± 1.3 versus 0.5 ± 0.57 in preterm labor normotensive group. ($p=0.001$)

Area under curve ROC for erythroblast count and predictive value of this test for pregnancy that subsequently complicated by preeclampsia is showed in Figure 1. Area under curve is 0.931 (confidence interval %95; range 0.885-0.976, P value=0.0001). Erythroblast count 1.5 is a point of convergence for prediction of pregnancy that subsequently complicated by preeclampsia or continue with normal blood pressure at delivery if mean of NRBC is <1.5. On the basis of 1.5 erythroblast with confidence interval %95, $P=0/0001$, there was specificity =93.02, NPV=98.16, accuracy=91.65 (table 2).

Table 1: demographic data of preeclamptic and normotensive groups.

Variable	Normotensive Group	Preeclamptic Group	P value
Number	549	50	0
Maternal age	26.15±3.30	27.04±4.31	0.076
Gestational age	39.07±0.94	37.58±1.46	0.009
Systolic BP	112.94±7.84	146.30±7.20	0.009
Diastolic BP	70.34±7.31	95.50±5.27	0.009
Birth weight at delivery	3248±312	2794±399	0.009
NRBC count	0.44±0.55	2.46±1.23	0.009

Table 2: statistical variable for accuracy of diagnosis of erythroblast in preeclampsia.

Confidence interval %95			
Variable	Frequency	Lower limit	Upper limit
Sensitivity	%61.54	%42.53	%77.57
Specificity	%93.02	%90.63	%94.83
PPV	%28.57	%18.42	%41.48
NPV	%98.16	%96.64	%99.00
Accuracy	%91.65	%89.86	%93.15

Discussion:

In this study erythroblast count in maternal blood was significantly higher in subsequently preeclamptic group. This is like other studies that shown elevated numbers of erythroblasts are present in the circulations of pregnant women with preeclampsia.^(30,31)

A significant proportion of the erythroblasts in the circulation of women with preeclampsia were of fetal origin. Authors showed perturbation in fetal-maternal cell traffic takes place earlier in pregnancy , before the symptoms of preeclampsia have become clear.⁽³²⁾ Other performed a prospective study in which the erythroblasts from blood samples drawn at around 20 weeks gestation were counted.⁽³³⁾ These results were then compared against the pregnancy outcomes to determine whether any correlation existed between erythroblast numbers and adverse pregnancy out-

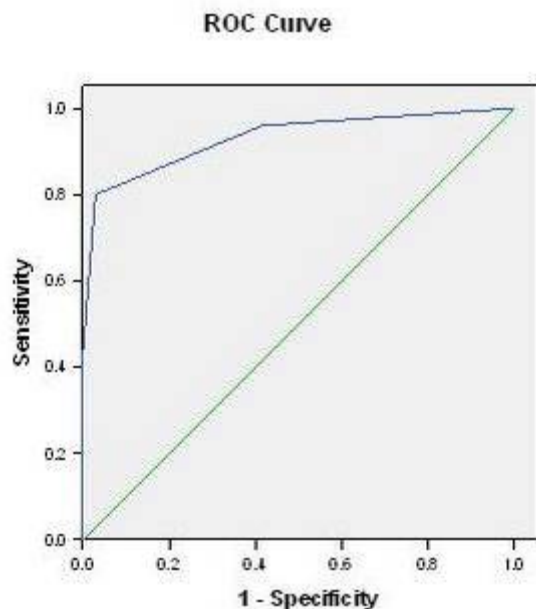


Figure1. Area under curve for erythroblast's count in prediction of preeclampsia

comes, particularly preeclampsia. Indeed there was a significant increase in the number of erythroblasts in peripheral blood samples taken during early pregnancy from women who subsequently had preeclampsia⁽³⁴⁾ this result is like our study.

Although we made no attempt to discriminate between erythroblasts of the fetal maternal origins in this study, but other results with both fluorescence in situ hybridization and single-cell polymerase chain reaction have demonstrated that a significant proportion of the erythroblasts in the maternal circulation are fetal⁽³⁵⁾. These new results therefore suggest that the traffic of fetal cells into maternal periphery is disturbed early in pregnancies subsequently complicated by preeclampsia⁽³⁶⁾. Because the primary pathologic mechanism of preeclampsia is suspected to be a defect in placentation, with the trophoblast unable to efficiently invade the deciduas and modify the maternal spiral arteries, our data suggest that these early events are also associated with increased leakage of fetal cells into the maternal periphery. In our study NRBC was significantly higher in maternal blood with IUGR and premature neonates, like Al-Mufti et al.^(13,14,30)

Although intrauterine growth restriction is frequently associated with pregnancies affected by preeclampsia⁽¹⁴⁾ no similar elevation in erythroblast numbers was detected in pregnancies complicated by intrauterine growth restriction but not complicated by preeclampsia. This results implies that fundamental differences exist between the etiologies of intrauterine growth restriction and preeclampsia and suggest

that placental dysfunction with associated disturbed fetal maternal cell traffic may be more specific feature of preeclampsia than of intrauterine growth restriction per se.^(30,37)In our study NRBC in IUGR with preeclampsia was significantly higher than group of IUGR without preeclampsia, increase in NRBC may be due to preeclampsia in this group.

The causes of preeclampsia remain unclear, but the underlying changes leading to the disorder occur early in pregnancy, before manifestation of the symptoms. Some described the elevation of the number of erythroblasts in pregnancies affected by preeclampsia.⁽¹⁷⁾ Studies performed on samples collected during the second trimester indicate that this disorder occurs early in those pregnancies in which preeclampsia subsequently develop^(21,15,33). Authors showed a higher number of NRBC in the blood of patients with PIH than in control samples, but the increase was not statistically significant ($p > 0.05$). Not all hypertension in pregnancy is caused by preeclampsia. Gestational hypertension without proteinuria or other systemic manifestations is frequently confused with preeclampsia but usually has a benign course⁽²⁵⁾. In one study, only 12 of the 30 pregnant women included in the group at risk for preeclampsia actually developed the disorder. They did not find statistically significant differences in the number of NRBC isolated from preeclampsia and from normotensive samples, but they observed a tendency for an enhanced number of NRBC in preeclampsia cases.⁽²⁵⁾

A serious clinical shortcoming is clearly represented by the fact that no reliable test exists to identify those women at risk for developing the disorder early enough in their pregnancies to permit preventive treatment. Current treatment is thus restricted to symptomatic management with drugs such as antihypertensives, benzodiazepines, or magnesium following full onset of the disease. Rapid delivery of the fetus often is the only way to resolve the disorder.

Whether we are now substantially closer to this long-awaited goal needs to be shown by appropriate screening studies for the prediction of preeclampsia, raising hope that in the near future we will be able to better deal with this dangerous pregnancy related disease.

Early recognition of patients at risk for development of preeclampsia /IUGR might allow a reduction of the worldwide mortality and morbidity associated with this complication of pregnancy. The availability of screening tests for early detection of this problem will help to identify patients in need of more careful monitoring and preventive treatment, to evaluate the progress of the disorder, and to improve pregnancy outcome.⁽³⁸⁾

Additional research will also be required to investigate whether abnormal fetal cell traffic of NRBC may be detectable even before the development of the clinical signs of preeclampsia. The presence of fetal erythroblasts in maternal blood, in combination with other biochemical markers, would be useful in screening for preeclampsia.

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