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Original Research Article

Significance of cord blood nucleated red blood cell count in pregnancies complicated by preeclampsia as a marker for intra uterine growth restriction

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ABSTRACT

Background: Preeclampsia is a pregnancy-specific syndrome of reduced organ perfusion secondary to vasospasm and endothelial activation which is typically characterized by hypertension, proteinuria, edema and fetal compromise which is a leading cause of intra uterine growth restriction (IUGR). Elevated nucleated red blood cell (NRBC) count is introduced as a potential marker of intra-uterine growth restriction (IUGR) hence determination of NRBC counts is essentially helpful in predicting short term neurodevelopment outcome. Objective of the study Elevated nucleated red blood cell (NRBC) count is introduced as a potential marker of intra-uterine growth restriction (IUGR) in term babies born to preeclamptic mothers
Methods: A cross sectional study conducted in a tertiary care hospital to evaluate the significance of cord blood NRBC count in term neonates born with pregnancy complicated by preeclampsia. It included 60 healthy mothers (control group) and 60 mothers with PIH. Collected data was analysed with SPSS software.
Results: The nucleated red blood cell in cord blood of newborns in preeclampsia group was significantly higher than in the control group (p value 0.013). IUGR is significantly higher in PIH group (p value 0.008).
Conclusions: From the observed data it is concluded that IUGR is an important cause of perinatal morbidity and mortality. The commonest maternal cause for IUGR was pregnancy induced hypertension. The other contributing factors were anemia, lack of awareness (unregistered, unbooked cases) among mothers, poor maternal nutrition and poor weight gain during pregnancy. Infants of preeclamptic women have higher nucleated red blood cell count at birth than control which means that preeclampsia may produce an erythropoietic response in the fetus. The positive correlation between cord nucleated red blood cell counts in preeclamptic patients and control group indicates that may be the hypoperfused placenta plays a role in this correlation.

Keywords: Cord blood nucleated RBC, Preeclampsia, Intra uterine growth restriction

INTRODUCTION

Preeclampsia (PE) is gestational proteinuric hypertension disorder, it is fundamentally related to poor trophoblatic invasion in the myometrium and this result in maternal spiral arteries hampered in their normal physiological vasodilatation.[1]

The production of erythrocyte in mammal is regulated by a humeral mechanism, the hormone erythropoitin, and depends up on the oxygen tension of tissue acting upon specific receptors.[2]

In human fetus elevated plasma erythropoietin levels have been found in high-risk pregnancies at delivery.

Because hypoxemia is the most important stimulus of erythropoietin synthesis and as the erythropoietin dose not cross the placenta, chronic intrauterine hypoxemia associated with intrauterine growth restriction is probably responsible for the elevated erythropoietin level in umbilical plasma. Erythropoietin increases in plasma after three to four hours from the beginning of moderate to severe hypoxemia and 48-72 hours before the appearance of NRBC in circulation.[3-5]

IUGR- It was defined as estimated fetal weight (EFW) or abdominal circumference (AC) less than 10th percentile of those born at the same gestational age or two standard deviations below the population mean for that gestational age.[6] Various maternal factors like vascular insufficiency, poor maternal nutrition, poor maternal weight gain during pregnancy are considered to be risk factors for IUGR. The growth restricted fetuses are at increased risk for respiratory distress, low Apgar score, necrotizing entero-colitis, hypoxic ischemic encephalopathy and other long term complications.[7]

Low birth weight (LBW) is another term used to define growth restricted babies but it includes preterm babies as well. The World Health Organization (WHO) definition of LBW babies is the babies weighing less than 2500 gm at birth.[8]

The Prenatal diagnosis of IUGR is based on clinical and ultrasonographic (USG) examination. USG is considered more accurate with less intraobserver variations. There are investigations studying the changes occur in the cells type or their distributive pattern under circumstances leading to IUGR. Disordered shift of nucleated red blood cells (NRBCs) has been noticed in specific pregnancy complications.[9,10]

Elevated NRBC count is in relation with complications like hypoxia, hypoxemia, asphyxia, maternal diabetes, prenatal brain damage, preterm infants, RDS, and preeclampsia. Increased NRBCs could itself provoke progressive growth restrictions. Therefore, NRBC count is introduced as a marker to show disturbed fetal status and could help detect and manage obstetric and neonatal complications prior to birth.

This study intends to investigate the disturbances of NRBC count in IUGR infants born to PIH mothers.

METHODS

This is a cross sectional study in order to compare the NRBC in the umbilical cord blood of term neonates born to 60 mothers with pre eclampsia and 60 healthy mothers studied over a period of 21 months from November 2017 to August 2019. The NRBC count was determined with laboratory procedures in the blood samples from umbilical cord of neonates. The hematologic factors were evaluated by collecting 2CC of the arterial blood of the umbilical cord, deposited in tubes containing ethylene di-amine tetra acetic (EDTA) and sent to the laboratory of the hospital for hematologic analysis.

Using an automatic hematologic blood cell counter, the number of WBC/mm^3 and the level of hemoglobin (g/dL) will be determined. Subsequently, blood smears on a glass slide will be prepared and stained with the Wright method; then the number of NRBCs (NRBC per 100 WBC) will be determined manually. Furthermore, the count of NRBC will be determined using the percentage of NRBC and the number of WBC per cubic milliliter blood of umbilical cord.

Inclusion criteria

All term babies born to woman admitted in view of pregnancy induced hypertension, with

- Blood pressure >140/90mmhg
- Proteinuria >300mg/dL in 24hrs
- Dipstick results >1+ in random samples.

Normal term new born babies.

Exclusion criteria

Mothers with the following condition

- Maternal diabetes mellitus, Chorioamnionitis

Babies with the following conditions

- Congenital anomalies
- Twin-to-twin transfusion
- Severe Anemia
- Cyanotic Heart Disease

RESULTS

The comparison of age between mothers in PIH group and Control group. There was no significant difference between the two groups with mean age of 24.37 in PIH group and 24.42 in control group (Table 1).

Table 1: Evaluation of age in both the groups.

	PIH group	Control group
Mean age	24.37	24.42
Std. dev	4.58	4.61
p value	0.95	
Range	18 to 39 years	18 to 39 years
Inference	Non significant difference in age when both the groups are compared	

In this study a total of 120 patients were enrolled in the study after satisfying the inclusion criteria and who gave informed consent. The study population was divided into 2 groups 60 in each group i.e test group defined as pregnant female having pregnancy induced hypertension

(PIH) and control group with mothers having normal pregnancy.

In the test group with pregnant females having PIH, the mean age was 24.37 years while that of control group was 24.42 years which was comparable as given in Table 1.

Majority of the pregnant females were in the reproductive age group 23-26yrs of age in both test and control groups. In the PIH group, 18 belonged to 18-21yrs of age, 27 were in 22-26yrs, 11 in 27-31yrs, 4 in 32-36yrs and 1 in >37 yrs of age while in control group 17 belonged to 18-21 yrs of age, 26 were in 22-26yrs, 12 in 27-31 yrs, 3 in 32-36 yrs and 1 in > 37 yrs of age as given in Table 2.

Table 2: The distribution of age among mothers between PIH and control group.

Age distribution	PIH group	Control group
18-21 years	17	18
22-26 years	27	26
27-31 years	11	12
32-36 years	4	3
37-40 years	1	1

Table 3: The incidence of NRBCs/100 WBC which is significantly higher in PIH group.

Significant N-RBCs	Present	Absent
PIH group	28	32
Control group	15	45
p value	0.013	
Inference	Significantly higher incidence of N-RBCs in PIH group	

Similarly Table 3 depicts number of neonates having significant number of nucleated RBCs was higher in PIH group when compared to normal group. The P value was significant at 0.013 with 28 neonates of 60 in PIH group having significant count of nucleated RBCs compared to 15 in control group.

Table 4: The incidence of IUGR between the two groups, with significantly higher incidence in PIH group (p value - 0.008).

IUGR	Present	Absent
PIH group	9	51
Control group	1	59
p value	0.008	
Inference	Significantly higher incidence of IUGR in PIH group	

As depicted, the incidence of IUGR was higher in neonates born to mothers with PIH. 9 neonates had IUGR in PIH group when compared to just 1 in the control group. The P value was significant at 0.008. Presence of PIH is a risk factor for development of growth restriction in neonates. PIH is associated with IUGR in neonates born to such mothers.

Table 5: Nucleated RBCs as predictor of IUGR in all the patients.

	NRBCs +	NRBCs -
IUGR+	8	2
IUGR-	35	65
Odd's ratio	7.4286	
95% CI	1.4952 to 36.9	
Z statistic	2.452	
p value	0.0142	
Inference	Presence of NRBCs is significant predictor of IUGR	

This table depicts the presence of IUGR in babies born with significant NRBC with odds ratio of 7.4286 a significant p value of 0.0142.

DISCUSSION

Neonates weight was expectedly lower in the IUGR group ($p<0.008$). We found that the NRBC count was significantly higher in the IUGR group ($p<0.013$). No other difference was found comparing the other variables including maternal age, gravidity numbers

It has been stated that the inability of cytotrophoblasts to differentiate correctly and subsequent failure to invade the uterus and its arterioles efficiently in preeclampsia lead to a relatively hypoxic placenta.[11] So compensatory mechanisms like enhanced production of erythrocytes (nucleated RBCs) are activated to counteract this imbalance. Maternal nucleated red blood cells have been studied in preeclampsia patients and have been shown to be raised compared to counts in maternal blood in controls suggesting disturbed fetomaternal cell trafficking in preeclampsia.

A low first minute APGAR score in newborn is also associated with high nucleated RBC level.[12] It has been observed that caesarean delivery for fetal distress, IUGR, oligohydramnios, low APGAR scores, and fetal academia (as indicated umbilical arterial pH <7) were associated with statistically significant increases in nucleated red blood cell counts

Neonates with higher NRBC counts were more likely to be admitted to the NICU according to the results of the present study. It also has been observed that NRBC levels tend to remain elevated for longer time during neonatal period among neonates with chronic fetal asphyxia compared to those babies who had acute fetal distress during delivery.

There are many investigations confirming the elevation of NRBC count in IUGR and other circumstances ending in IUGR or several other fetal abnormalities. Those data

is always said to be due to the increased erythroblasts in fetal circulation even if placenta has normal perfusion.[12] However, Vatansever U et al, reported significant relation between NRBC count and neonatal relative hypoxia in IUGR, asphyxia, diabetic mothers, and preterm infants. There was not such an association in case the serum erythropoietin was measured.

They suggested that NRBC count could have predictive value in short term neurodevelopmental outcome or in IUGR complications.[13]

Fetal hypoxemia can trigger erythropoietin release that causes stimulation of red blood cells, both at intramedullary and extramedullary sites. In the human fetus, erythropoiesis typically progresses from erythroid commitment of colony forming stem cells to extrusion of nuclear material with concomitant reduction in cell size.

This process yields a mature red blood cell without a nucleus that contains the highest concentration of hemoglobin. Early stages of mature erythropoiesis typically are confined to the bone marrow where capillary fenestrations limit the passage of large NRBC precursors into the peripheral circulation. Conversely, extramedullary sites are believed to have larger capillary fenestrations that permit the release of large NRBC's .During periods of high extra medullary production. NRBC counts upto 30/100WBC's are physiologic at <30 weeks of gestation, although levels of 5-10/100 WBC are normal thereafter.

Isolated polycythemia in intrauterine growth restricted fetuses suggests enhanced intramedullary erythropoiesis, polycythemia with elevated NRBC count is suggestive of chronic extramedullary hematopoiesis.

The NRBC count in the peripheral circulation of growth restricted neonates has received special interest as a surrogate marker for the severity and chronicity of fetal acid base disturbance and a prognostic factor for adverse outcome.

CONCLUSION

Preeclampsia is considered to be a state of deterioration of utero-placental perfusion. Under such condition of lowered oxygen tension, it is feasible that compensatory mechanism will activate to counteract this imbalance for example, the enhanced production of erythrocytes, which could account for the increase in erythroblasts Nucleated RBC are precursors of red blood cells, released from fetal bone marrow in response to increased erythropoietin caused by hypoxia, in term neonates is an indicator of chronic intrauterine hypoxia.

As erythropoietin does not cross the placenta, elevated concentrations in fetal cord blood in cases of hypoxaemia are of fetal origin

In this study authors found that the average number of NRBC count/100 WBCs was and is significantly higher in PIH group than in control group. As described, this number could be reliably considered as fetal NRBC count. Increased NRBC could have a predictive role in IUGR diagnosis and help in assessing fetal distress before birth and before ultrasonic diagnosis, and could help in early intervention.

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