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Original scientific paper

Nucleated Red Blood Cells Count as First Prognostic Marker for Adverse Neonatal Outcome in Severe Preeclamptic Pregnancies

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A B S T R A C T

The purpose of this study was to determine acceptability of the nucleated red blood cells counts (NRBC) as early prognostic parameter for adverse outcome in preterm neonates born from pregnancies complicated with severe preeclampsia. We analysed 77 premature newborns who were born from pregnancies with severe preeclampsia during eight years (2004–2011) in our tertiary center. Women with other pregnancy complications were excluded from the study, as well as newborns with malformations and chromosomal anomalies. Newborns were compared according to the count of nucleated red blood cells (NRBC) on the first day of life. Cut off of NRBC was determined at 40 per 100 white blood cells. We analyzed and compared birth weight, gestational age, Apgar scores in 1st and 5th minute, hypoglycemia in first day of life, need for respiratory support, neonatal infection and brain ultrasound findings at the day of discharge between the groups of newborns. We found significantly lower birth weight, gestational age and Apgar scores in case group (NRBC> 40) and significantly higher rate of infections, need for respiratory support, abnormal brain ultrasound findings, morbidity rate and adverse neonatal outcome compared to control newborns group. Increased count of nucleated red blood cells (NRBC) in preterm newborns born from pregnancies with severe preeclampsia seems to be the first significant marker for detecting adverse neonatal outcome.

***Key words**: nucleated red blood cells, preeclampsia, newborns, perinatal outcome*

Introduction

Preterm delivery accounts for 80% of perinatal mortality and more than half of the long term morbidity[1]. Common causes of iatrogenic preterm delivery are related to severe maternal complications such as preeclampsia, placental abruption, intrauterine growth restriction or fetal distress. During the last decades survival of preterm newborns has increased considerably, but still with increased incidence of neurodevelopmental impairments, respiratory, visual and hearing disturbances. Identifying acceptable prognostic markers to detect adverse perinatal outcome is of a major interest of perinatologists and neonatologists[2,3]. Placental hormones have been investigates as biochemical markers of obstetric diseases and ultrasound markers have been joined in recent years[4]. Many studies have been performed on the use of favourable predictive markers for the early prevention of both, preeclampsia and preterm delivery and the perinatal outcome, although results have been poor so far[4–6]. Nucleated red blood cell (NRBC), a premature red blood cell, is an indicator of hematopoiesis in a newborn infant and has been known to be associated with intrauterine hypoxia. An increase in NRBC count at birth has been known to be attributable to fetus hemorrhage, preterm pregnancy, intrauterine growth retardation, diabetes mellitus, Rh immunisation, and preeclampsia. In addition, as a prognostic factor of perinatal complications, NRBC count has been known to be closely associated with bronchopulmonary dysplasia, intraventricular hemorrhage, necrotizing enterocolitis, and death[7–9].

NRBC are primarily produced in fetal bone marrow in response to erithropoietin and stored in the marrow as precursors to reticulocytes and mature erythrocytes[10].

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The hematopoietic system responds to hypoxia by compensatory response; increasing erythropoietin that induces exaggerated erythropoesis, resulting in the release of immature red blood cells – NRBC in fetal circulation[7,10]. Many acute and chronic stimuli causes increase in circulating NRBCs from either increased erythropoietic activity or a sudden release from the marrow storage pools[10]. Increase of NRBC counts in fetal hypoxia brought out the consideration of using the NRBC counts as a marker for hypoxia and possible predictor of adverse outcome of affected newborn.

The course of severe preeclampsia is associated with a progressive deterioration of the mother[11]. Preeclampsia is characterized by endothelial damage, platelet activation and intravascular coagulation that may lead to poor placental perfusion and therefore to fetal hypoxia and growth restriction[4,12]. Such pregnancies are terminated when maternal and fetal conditions are altered; delivery is the only treatment for the mother but results in birth of premature newborn, often growth restricted and with increased risk of adverse outcome[10–13].

The purpose of this study was to determine the possible corelation of NRBC counts and severity of condition and adverse outcome in preterm newborns born from severe preeclamptic pregnancies.

Patients and Methods

Our study analyzed 77 premature newborns of less than 37 weeks of gestation, born at a tertiary care University hospital from pregnancies complicated with severe preeclampsia during a period of eight years (2004–2011). Diagnosis of severe preeclampsia was defined as a presence of 1 of the following symptoms or signs in the presence of preeclampsia: blood pressure >160/110 mm Hg, proteinuria >5 g/24 h, oliguria <400 mL/24h, pulmonary oedema or cyanosis, persistent headaches, epigastric pain/or impared liver function, thrombocytopenia.

We excluded pregnancies with premature rupture of membranes, infections and chorioamnionitis, Rh immunisation, diabetes mellitus and other pathology that could affect the condition of the newborn and additionally influence the nucleated red blood cell counts in newborn. Newborns with chromosomal abnormalities and congenital malformations were also excluded from the study.

We compared newborns from severe preeclamptic pregnancies according to the count of nucleated red blood cells (NRBC) from vein sample within 12 hours after birth. The count of NRBC was performed using the automated hematology analyzer as NRBC *per* 100 white blood cells (WBC) with cut off at 40 *per* 100 WBC.

Birth weight (BW), gestational age (GA), Apgar scores at 1st and 5th minute, need for respiratory support, supplementary oxygen, mechanical ventilation and duration of stay in incubator were analyzed and compared according to NRBC count. Hypoglycemia at the day of birth (< 2 mmol/L) and early or late onset of infection (according the number of WBC, band neutrophiles, CRP, blood culture, the conditions of infants) were also analyzed. All infants included in the study had a brain ultrasonogram at the day of discharge and periventricular leukomalacia (PVL), intraventricular hemorrhage grade 3 (IVH3), posthemorrhagic hydrocephalus and intraparenchymal lesions (IPL) were marked as pathologic findings. Neonatal outcome in means of chronic lung disease (CLD) or death were analysed as well. Data were statistycaly analyzed using Mann Whitney test and Chi-square test. $p<0.05$ was considered significant.

Results

A total of 77 newborns were included in the study. All of them were premature and born from mothers with severe preeclampsia. Five neonates (6.5%) had extremely low gestation age, 23 (29.9%) had 28–30 weeks of gestation, 21 (27.3%) had 30–32 weeks of gestation and 28 of them (36.3%) had 33 to 36 weeks (Table 1). 16 newborns (20.8%) were extremely low birth weight, 28 (36.4%) newborns had 1000–1500 g and 33 (42.8%) were above 1500g (Table 1). 56 newborns had NRBC count <40 (Group 1) and 21 newborns had NRBC count >40

TABLE 1
GESTATION AGE OF PRETERM NEONATES

GA (weeks)	N	%
<28	5	6.5
28–30	23	29.9
30–32	21	27.3
33–36	28	36.3
BW (grams)		
<1000	16	20.8
1000–1500	28	36.4
>1500	33	42.8

N – number of newborns, GA – gestational age,
BW – birth weight

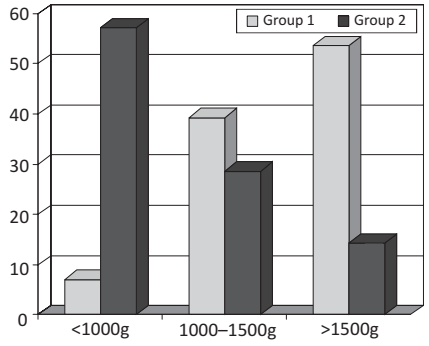

Fig. 1. Comparison of neonatal birth weight between the groups, p<0.0001. NRBC – nucleated red blood cells, g – grams, WBC – white blood cells. Group 1: <40 NRBC /100 WBC; group 2 : >40 NRBC / 100 WBC.

TABLE 2
COMPARISON OF GESTATIONAL AGE, APGAR SCORES AND BIRTH WEIGHT BETWEEN THE ANALYZING GROUPS

	Group 1 (N=56)	Group 2 (N=21)	p
GA (weeks)	32.3±2.19	29.67±1.9	<0.0001
BW (grams)	1577.59±449.51	1071.9±286.8	<0.0001
Apgar 1st minute	6.75	5.38	0.028
Apgar 5th minute	8.32	7.24	0.026

Group 1: <40 NRBC / 100 WBC; Group 2 : >40 NRBC / 100 WBC
N – number of newborns, GA – gestational age, BW – birth weight, NRBC – nucleated red blood cells, WBC – white blood cells, $\bar{X}$±SD

TABLE 3
COMPARISON OF NEONATAL COMPLICATIONS BETWEEN THE GROUPS

	Group 1 N=56	Group 2 N=21	p
Duration of stay in incubator (days) ($\bar{X}$±SD)	24.4±2.05	45.95±6.45	<0.002
Duration of oxygen dependancy (days) ($\bar{X}$±SD)	9.18±1.6	35.8±6.92	<0.0001
Duration of mechanical ventilation (days) ($\bar{X}$±SD)	0.46±0.22	16±6.05	<0.0001
CLD (number)	4	8	0.001
Hypoglicaemia (number)	20	15	0.001

Group 1: <40 NRBC/100 WBC; Group 2 : >40 NRBC/100 WBC
CLD – chronic lung desease, NRBC – nucleated red blood cells, WBC – white blood cells

(Group 2). We found significant difference in low birth weight (p<0.001) and significant difference in gestational age (p<0.0001) between the groups (Table 2). Distribution of birth weight (BW) and gestational age (GA) of newborns are presented in Figure 1 and Figure 2.

Apgar scores in 1st and 5th minute were significantly lower in case group comparing with the control group (p=0.028 and p=0.026 respectively, Table 2). Duration of stay in incubator was significantly longer in case group, respiratory support in terms of duration of oxygen dependancy, duration of mechanical ventilation and chronic lung disease were also significant (p<0.0001, Table 3). There were significantly higher rates of infections (Figure 3) and hypoglycemia (p<0.0001) between the groups (Table 3). We found higher incidence of brain ultrasonogram findings of IVH3 and IPL in case group (p=0.015, Table 4) as well as significant difference in adverse neonatal outcome in case group (Figure 4).

Analyzing the influence of birth weight and gestational age to newborns from the case group we found only a significance in number of days spent in incubator (Table 5).

TABLE 4
COMPARISON OF NEONATAL BRAIN ULTRASOUND FINDINGS AT THE DAY OF DISCHARGE

	Group 1 N	Group 2 N	p
Normal	42	10	<0.049
IVHgr III/IPL, posthemorrhagic hydrocephalus	2	5	<0.02
PVL grIII	11	6	<0.61

Group 1: <40 NRBC/100 WBC; Group 2 : >40 NRBC/100 WBC
NRBC – nucleated red blood cells, WBC – white blood cells, N – number, PVL – periventricular leukomalacia, IVHgr III – intraventricular hemorrhage grade 3, IPL – intraparenchymal lesion, PVL gr 3 – cystic periventrikular leukomalatio

Discussion and Conclusion

The incidence of severe preeclampsia less then 36 weeks is 0.3%[9]. Women with severe preeclampsia are usually delivered promptly to prevent maternal and fetal complications. The risk of prolonging pregnancy is worsening maternal endothelial dysfunction and continued

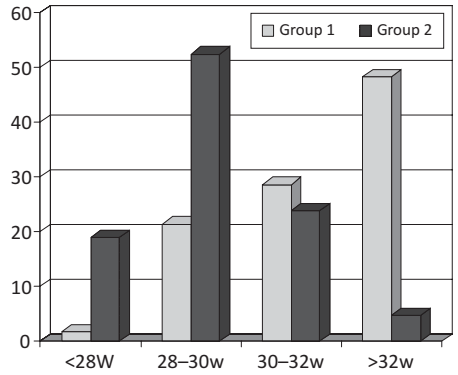

Fig. 2. Comparison of neonatal gestational age between the groups. p<0.0001. NRBC – nucleated red blood cells, w – weeks, WBC – white blood cells. Group 1: <40 NRBC/100 WBC; group 2 : >40 NRBC/100 WBC.

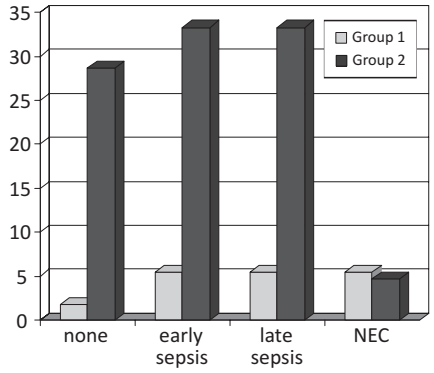

Fig. 3. Comparison of infections rate in newborns between the groups, p<0.0001. NEC – necrotic enterocolytis. Group 1: <40 NRBC/100 WBC; group 2 : >40 NRBC/100 WBC.

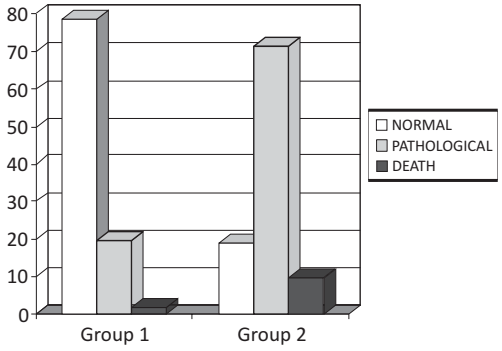

Fig. 4. Outcome of newborns born from mother with severe preeclamptic pregnancies according NRBCs count, p<0.0001. Group 1: <40 NRBC/100 WBC; group 2 : >40 NRBC/100 WBC. NRBC – nucleated red blood cells, WBC – white blood cells

poor perfusion of major maternal organs with the potential for severe end organ damage to the brain, kidneys, liver, hematologic system, vascular system and placenta with consequent risk of seizures, pulmonary edema, hypertensive encephalopathy, stroke, renal and hepatic failure, retinal detachment, blindness, disseminated intravascular coagulation, placental abruption and death[12,13]. Newborns from preeclamptic pregnancies are at high risk of short-term complications and long-term disability. Fetal and neonatal consequences include preterm birth, stillbirth, growth restriction and admission to a neonatal intensive care unit. However, according Bombrys et al., the limits of viability vary among hospitals and are impacted by factors such as birthweight and gestational age [13]. We found in our study significant difference in these parameters, too (Table 2). Preeclampsia does not appear to accelerate fetal maturation, as once believed[13]. Currently, there are no clinically available tests that perform well in distinguishing women who will develop preeclampsia from those who will not. So, the purpose of the post-diagnostic evaluation is to determine the severity of disease and assess maternal and fetal well-being.

It is known that preeclampsia leads to increased number of NRBC in newborns as a reflection of chronic

TABLE 5
COMPARISON OF GESTATIONAL AGE, BIRTH WEIGHT AND APGAR SCORE AS INDEPENDENT PROGNOSTIC FACTORS AND THEIR IMPACT TO NEONATAL OUTCOME IN CASE GROUP

Parameters	BW	GA	APGAR score
APGAR 1st min.	NS	NS	/
APGAR 5th min.	NS	NS	/
pH	NS	NS	NS
BE	NS	NS	NS
Hypoglicaemia	NS	NS	NS
Infections	NS	NS	NS
Ultrasound findings	NS	NS	NS
CLD	NS	NS	NS
Incubators (days)	p=0.02	p=0.04	NS
Outcome at discharge	NS	NS	NS

CLD – chronic lung desease; NS – non significant, BW – birth weight, GA – gestational age, BE – base excess

hypoxia[14] but we found insufficient data investigating correlation of fetal NRBC counts and severity of perinatal outcome in such illness. Prognostic value of NRBC in prematures is still considered limited[1,6]. We tried to emphasize the value of NRBC counts in preterm infants born from severe preeclampsia as a early prognostic marker for adverse neonatal outcome. Our results found that newborns in case group had significantly lower Apgar scores, birth weight and gestational age (Table 2, Figure 2, Figure 3), had longer stay in intensive care unit (Table 3), had significantly higher rates of infection (Figure 3), hypoglycemia (Table 3) and significantly often abnormal ultrasound findings (Table 4).

During the last decades survival of preterm newborns has increased considerably, but they still have an increased incidence of neurodevelopmental imapairments, gastrointestinal and respiratory complications[15]. Investigations has been made whether the NRBC count in premature newborns can predict severity of brain white matter injury[16–21]. The results were inconclusive. Our research of newborns born from severe preeclamptic pregnancies did not notice significant diference in white matter damage. We found higher rates in IVH gr III, intraparenchimal lesion and posthemorhagic hydrocephalus in case group (Table 4).

Our data indicates that within the newborns with NRBC count >40 / WBC, neither birth weight alone or gestational age, individually determinate adverse neonatal outcome (Table 5).

The mechanisms regulating fetal growth and development are disrupped in preeclampsia, only further investigations in better understanding of the pathophysiology of the disorder may allow us to develop strategies to prevent morbidities from fetal through adult life. Because of the high variability of each case, a general recommendation for the optimal timing of delivery is not possible[22–25]. Our data indicate that high NBRC count in preterm

preeclamptic neonates could implicate adverse neonatal outcome, regardless of birth weight and gestational age (Figures 3 and 4). Consenquently, the increased count of nucleated red blood cells at birth in newborns from severe preeclamptic pregnancies can be first and relevant independent predictor of adverse neonatal outcome.

Our results strongly suggest that NRBC count >40/100 WBC in preterm neonates born from pregnancies complicated with severe preeclampsia demonstrate serious independent prognostic factor for adverse neonatal outcome.

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ERITROBLASTI KAO NOSIOCI MORBIDITETA U NOVOROĐENČADI IZ PREEKLAMPTIČKIH TRUDNOĆA

SAŽETAK

Cilj ovog istraživanja bio je usporediti ishod novorođenčadi rođenih iz preeklamptičkih trudnoća prema broju eritroblasta. Analizirali smo 77 nedonoščadi iz trudnoća s teškim preeklampsijama rođenih u našem tercijarnom centru tijekom perioda od 8 godina. Žene s drugim komplikacijama trudnoće i poremećajima koji bi mogli dodatno utjecati na ishod isključene su iz istraživanja kao i novorođenčad s malformacijama i kromosomskim anomalijama. Usporedili smo novorođenčad prema broju eritroblasta po porodu. Rezni broj eritroblasta utvrđen je na 40 eritroblasta na 100 leukocita. Analizirani su Apgar ocjena u 1. i 5. minuti, hipoglikemija u prvom danu života, respiratorna potpora, infekcija, nalaz ultrazvuka mozga kod otpusta te ishod djeteta. Ustvrdili smo značajno nižu porođajnu težinu, gestacijsku dob i Apgar ocjenu u novorođenčadi s većim brojem eritroblasta, kao i njihovu značajno veću sklonost infekciji, patološkim nalazima ultrazvuka mozga kao i značajno lošijim ishodom. Novorođenčad s povišenim brojem eritroblasta pokazuje značajno povišen morbiditet. Povišeni broj eritroblasta 12 sati po porodu u novorođenčadi iz preeklamptičkih trudnoća može biti prvi pokazatelj mogućeg nepovoljnog ishoda u ovoj visoko rizičnoj grupi novorođenčadi.