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CLINICAL STUDY

# Impact of asphyxia on red blood cell folate concentration levels in newborns

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**ABSTRACT**

OBJECTIVES: To determine whether red blood cell (RBC) folate concentration levels are correlated with the occurrence of neonatal asphyxia and to study the effects of gestational age, gender, and mode of delivery on RBC folate concentration levels in newborns.

BACKGROUND: Asphyxia is one of the frequent causes of morbidity and mortality of newborns. Severe perinatal asphyxia can arise due to many factors.

METHODS: In a prospective study, the RBC folate concentrations were determined on day 1 of life in the whole group (n = 181) of full-term (n = 121) and preterm (n = 60) newborns. Immunochemical analysis for the determination of folate in erythrocytes was performed.

RESULTS: RBC folate concentration levels in asphyxiated newborns (n = 16) were significantly decreased (median 974 ng/ml; p = 0.023) in comparison with healthy newborns. On the other hand, the RBC folate concentration levels were significantly increased in preterm newborns (median 1,212 ng/ml; p = 0.01) in comparison with full-term newborns (median 1,098 ng/ml). Higher RBC folate concentration levels were found in newborns which had been delivered by Caesarean section (median 1,188 ng/ml; p = 0.02) compared to those born vaginally (median 1,098 ng/ml).

CONCLUSION: Our results confirmed a significant decrease in RBC folate concentration in asphyxiated newborns on their first day of life *(Fig. 4, Ref. 36)*. Text in PDF *www.elis.sk.*

KEY WORDS: asphyxia, preterm newborn, full-term newborn, delivery, red blood cell folate.

**Abbreviations:** RBC – red blood cell, EDTA – ethylenediaminetetraacetic acid, MTHFR – methylenetetrahydrofolate reductase

## Introduction

Asphyxia is one of the most frequent causes of morbidity and mortality in neonates. Severe perinatal asphyxia can arise due to many factors on the side of both the mother and the newborn.

Perinatal asphyxia is still a health concern worldwide. It can result from many factors. Maternal malnutrition can also increase the risk of perinatal asphyxia and is a major cause of neurologic damage (1). If persistent, perinatal asphyxia is a condition of impaired blood gas exchange that leads to progressive hypoxemia and hypercapnia with metabolic acidosis (2). Perinatal asphyxia interferes with neonatal development and results in long-term deficits associated with mental and neurological diseases with delayed clinical onset (3) including severe mental retardation, spastic quadriplegia, microcephaly, seizures, and sensory impairment. The degree of abnormality determined upon neonatal examination, electroencephalogram, and neuroimaging studies predicts the neurodevelopmental outcome (2).

Higher prevalence of perinatal asphyxia was documented in male sex and in African-American race (4). Perinatal asphyxia occurs in up to 25 % of infants of diabetic mothers. It may result from prematurity, Caesarean delivery, intrauterine hypoxia caused by maternal vascular disease, or macrosomia (2).

During perinatal asphyxia, the primary insult relates to the duration of the period of lacked oxygenation and leads to death if not re-established. Re-oxygenation leads to a secondary insult related to a cascade of biochemical events required for restoring proper function (3). Strong hypoxia might lead to the apoptosis induction (5).

There is no specific blood test to diagnose perinatal asphyxia. Some of biochemical indices are metabolic acidosis with pH < 7.0 and base excess < 20 mEq/L (2). Some authors applied heart markers as early indicators and prognostic parameters of perinatal asphyxia (6). Also some markers of oxidative stress can be decreased during perinatal asphyxia. The increase in total anti-

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oxidant capacity in asphyxiated term newborns could take place based on certain ability of asphyxiated term newborns to prevent the damage of balance between overproduction of malondialdehyde and antioxidants (7). Malondialdehyde is one of the oxidative stress indicators in human body. After folate supplementation, a significant malondialdehyde decrease was observed in colostrum and mature human milk (8). In combination with clinical signs of asphyxia and other biochemical blood parameters it is possible to predict severity of the asphyxial insult (9).

Folate-mediated one-carbon metabolism is essential for metabolic processes in the body (10). Interference of abnormal metabolism in the pathogenesis of neurological and vascular diseases has been published (11). The importance of folates and other nutrients during critical stages of pregnancy is currently recognized. The exposure to epigenetic changes is responsible for altering the mechanisms of growth and metabolism and leads to changes that may persist throughout life causing an increased susceptibility to disease. Some factors before and after birth can decrease levels of stress (12). Risk factors associated with birth defects of central nervous system include deficiency in folate supplementation, cigarette smoking, and exposure to x-rays (13). In developing countries, folic acid and iron supplementation of pregnant women could be beneficial to prevent children's malnutrition. Folic acid participates in cardiovascular prevention (14).

Adequate nutrition which was recorded in the reduction in hyperhomocysteinemia is an important factor in prevention of cardiovascular diseases (15). The risk of stable ischemic heart disease increases due to hyperhomocysteinemia. Slovak patients with stable ischemic heart disease were found to yield high prevalence of hyperhomocysteinemia (16).

The study is aimed at determining whether red blood cell (RBC) folate concentration levels are correlated with the occurrence of neonatal asphyxia and at studying the effects of gender on RBC folate concentration levels in newborns.

## Materials and methods

The study comprised newborns (n = 181) hospitalized at the Department of Neonatology in Children´s University Hospital in Bratislava between 2010 and 2012. Blood samples from umbilical cord were collected on their first day of life. Full-term newborns (group A: n = 121; sub-group $A_1$: healthy: n = 117; sub-group $A_2$: asphyxiated: n = 4; gestational age 38 to 41; median of birth weight 3450 ± 750 grams) and preterm newborns (group B: n = 60; sub-group $B_1$: healthy: n = 48; sub-group $B_2$: asphyxiated: n = 12 gestational age 27 to 37; median of birth weight 1600 ± 850 grams) were included in this study. All newborns with hemolytic disease of the newborn, multiple congenital anomalies, and severe bleeding with blood losses over 2 g/dl/24 hours were excluded. Perinatal asphyxia was defined by metabolic or mixed acidemia (pH < 7.00) in umbilical cord arterial blood sample, persistence of Apgar score below 4 points in 5th minute of life, and presence of neurological symptoms (2).

Blood samples for determining RBC folate were collected in vacutainers containing EDTA and taken to the Department of Clinical Biochemistry in Hospital for Children in Bratislava. Within 24 hours of collection, full blood count analysis (especially hematocrit) was undertaken. Remaining blood was stored at –20 °C for up to one month according to specifications of the producer sets and consistent with literature data (17). The blood samples were defrosted and processed within one month. The lysing agent (1.5 ml 0.2 % ascorbic acid) was added to 50 µl of whole blood for RBC folate analysis *(Roche Diagnostics, Germany)*. Immunochemical analysis was performed with the use of chemiluminescent emission (analyser *cobas e 411*). It was based on a competitive test using natural folate binding protein specific for folate. Folate competes with added folate for the binding sites of folate binding protein labeled with ruthenium complex.

Statistical analyses were based on frequency analysis calculated at 97 % accuracy with confidence limits for proportion. Analysis of median values for RBC folate concentration levels was done by Mann–Whitney or Kruskal–Wallis tests. The relationship between both groups of asphyxiated and non-asphyxiated newborns was assessed by Kendall rank correlation coefficient. The same analyses were performed in comparison with gender (male, female), gestational age (preterm, full-term newborns) and mode of delivery (spontaneous, Caesarean delivery). Differences were regarded as statistically significant at $p < 0.05$. Analyses were performed by *statsdirect* version 2.8.0.

The study was approved by the Ethics Committee of University Hospital in Bratislava and the Ethics Committee of Children´s University Hospital in Bratislava. Informed consent was obtained from every enrolled newborn's mother.

## Results

RBC folate concentration levels in asphyxiated newborns (n = 16) were significantly decreased (median 974 ng/ml; p = 0.023) (Fig. 1) in comparison with healthy newborns. Based on the low number of patients in each sub-groups ($A_1$, $A_2$, $B_1$, $B_2$), we did not analyze these results statistically.

The decreased RBC folate concentration levels are not associated with gender (male:female / 1:1.15) (Fig. 2). The median of red blood cell folate concentration of males was 1,167 ng/ml and that of females 1.127 ng/ml (p = 0.67).

On the other hand, the RBC folate concentration levels were significantly increased in preterm newborns (median 1,212 ng/ml; p = 0.01) (Fig. 3) in comparison with full-term newborns (median 1,098 ng/ml). In the group of asphyxiated newborns, 75 % were identified as preterm newborns.

The other impact on RBC folate concentration levels was the mode of delivery. The study group comprised newborns born by Caesarean section due to complications on the maternal or newborn's side or due to placental abnormalities. Higher RBC folate concentration levels were found in newborns which were born by Caesarean section (median 1188 ng/ml; p = 0.02) (Fig. 4) compared to those born vaginally (median 1098 ng/ml).

## Discussion

According to the American Academy of Pediatrics and the American College of Obstetricians and Gynecologists, perinatal as-

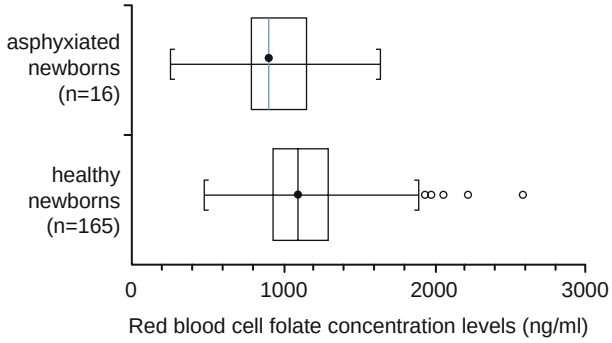


**Fig. 1. Red blood cell folate concentration levels in asphyxiated and healthy newborns on the first day of life (p = 0.023).**

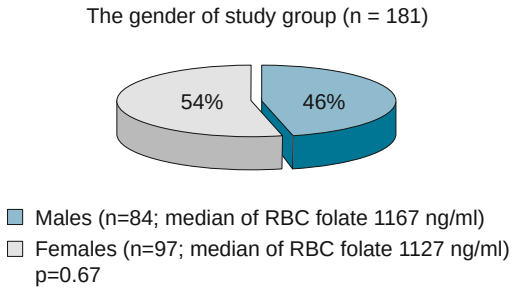


**Fig. 2. The findings of red blood cell (RBC) folate concentration levels due to gender in our study group (p = 0.67).**

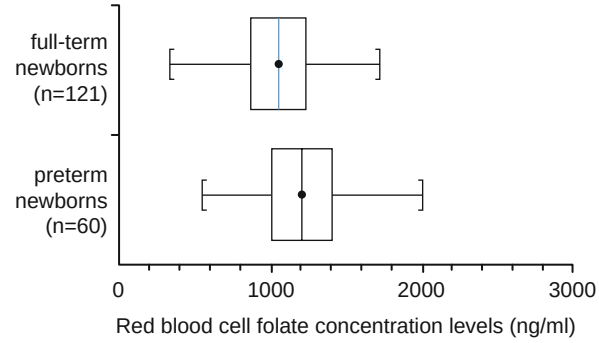


**Fig. 3. Red blood cell folate concentration levels in preterm newborns in comparison with full-term newborns (p = 0.01) on the first day of life.**

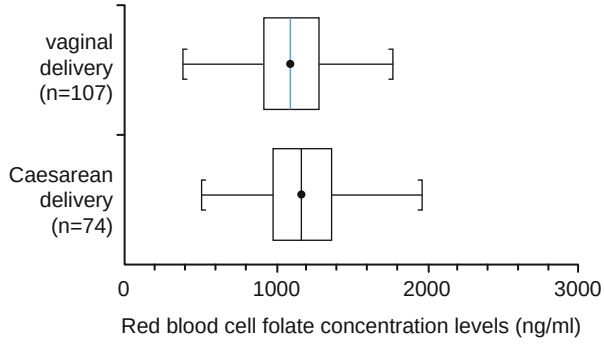


**Fig. 4. Impact of different mode of delivery on red blood cell folate concentration levels in newborns from umbilical cord blood samples (p = 0.02).**

phyxia should be present by profound metabolic or mixed acidemia (pH < 7.00) in umbilical cord arterial blood sample; persistence of Apgar score of 0–3 for > 5 min; neurologic manifestations in the immediate neonatal period includes seizures, hypotonia, coma, or hypoxic-ischemic encephalopathy; evidence of multiorgan system dysfunction in the immediate neonatal period (2). Asphyxia is one of the risk factors which have been identified in cerebral palsy (18). Maternal infections and prematurity are also important risk factors for birth asphyxia mortality. Premature infants are at higher risk of birth asphyxia mortality compared to term infants (19). In our study we identified 63 % of asphyxiated newborns as being born prematurely. Other factors such as abnormal fetal heart rate, prior Caesarean delivery, maternal age of 35 years or older were detected as independent risk factors of severe neonatal acidosis, which can lead to perinatal asphyxia (20).

The red blood cell folate is the primary indicator to determine folate adequacy. Because folate is taken up only by the developing erythrocyte in the bone marrow and not by the circulating mature erythrocyte during its 120-day lifespan, erythrocyte folate concentration is an indicator of long-term status (21). The normal range of RBC folate values vary due to the different analytical methods used for assessing RBC folate concentrations (22). The best indicator of long-term folate levels in the body is the RBC folate concentration level. Differences were observed in breastfed infants independent of maternal folate status which suggested that concentration of folate decreases from birth up to 24 months (23). The significant influence of other factors on RBC folate concentration levels which had been noted were e.g. climate, race (24) or smoking habits (25). The genetic polymorphisms of the MTHFR (methylenetetrahydrofolate reductase) can be also associated with some disorders such as major depressive disorders (26). Different climate and nutritional habits can have influence on prevalence of congenital anomalies as e.g. cleft palate in some regions of European countries (27, 28). It can alter the RBC folate concentration levels in organism. Decreased serum folate levels are correlated with the occurrence of neonatal asphyxia (29). The results of our prospective study were similar to those of Mi et al (2008). RBC folate concentration levels were not impacted by gender in our study. Laboratory findings associated with perinatal asphyxia pointed out the changes in blood count parameters such as erythrocyte count and hemoglobin and hematocrit values (9). On the other hand the chronic long-term intermittent hypoxia leads to an increase in haematocrit and whole blood viscosity (30).

One of further determinants of folate levels can be the gestational age. Some authors suggest the association between low maternal serum folate and risk of preterm birth (31). Joythi et al did not find differences due to gestational age (32). The placental transfer of vitamins can vary according to gestational age and body reserves. We confirmed higher RBC folate concentration levels in preterm newborns in comparison with the group of full-term

newborns, while the difference was statistically significant. We believe that an interaction between other substances in metabolism pathways has taken place, which might have led to raised RBC folate concentration levels. One of probable factors could lie in good maternal folate status during pregnancy. The maternal status of group B vitamins during pregnancy can be lower (33) and the impact on newborns is uncertain. Additional studies are required to elucidate the subsequent effects.

On the other hand the clearly positive effects of folic acid in prenatal and neonatal period were published in many studies. Nevertheless, excessive doses of folic acid could present the risk of accumulation and possible adverse effects (34).

Red blood cell folate concentration tended to change based on many factors. Mode of delivery, e.g. cesarean delivery could avert intrapartum fetal trauma and asphyxia and allow timed delivery to assure readiness of neonatal intensive care resources. Asphyxia can lead to changes in blood count parameters (9) and probably in RBC folate concentration levels as confirmed by Mi et al (29) as well as by the results of our study. A lot of retrospective and observational studies do not support routine cesarean delivery for all early preterm infants. Cesarean delivery may offer survival advantage to the periviable growth-restricted infant, regardless of fetal presentation and appears to offer survival benefit to the malpresenting fetus (35). The mode of delivery can influence the RBC folate concentration levels. We believe there is an impact resulting from certain mechanical injuries, but it is necessary to know the level of bilirubin. This could be due to the lack of research in our study, as the level of bilirubin was not studied in umbilical cord blood samples of our newborns. On the other hand, it is the confirmation of the presence of physiological changes during vaginal delivery that suggests that RBC folate concentration levels are very similar to the findings in a group of healthy full-term newborns in our country (36). During asphyxia, the RBC folate concentration levels decreased in spite of physiological blood count parameters (unpublished data).

## Conclusion

Folates are important parts of some metabolic processes in the human body in the first months of life. The analysis of its concentration in erythrocytes is an accurate indicator of the body's saturation with folic acid. RBC folate concentration is influenced by several factors on the side of both the mother and the newborn and/or other associated complications. In our prospective study we confirmed a significant decrease in RBC folate concentration levels in umbilical cord blood samples in asphyxiated newborns on their first day of life. Most of these asphyxiated newborns were born premature. With the increase in gestational age the RBC folate concentration levels decreased. Another factor which could have had an impact on RBC folate concentration levels detected by us was the mode of delivery. The decreased RBC folate concentration levels are not associated with gender. It can be due to other factors such as the size of selected group or division into specific sub-groups. This issue needs to be studied in greater depth.

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