Role of Nucleated Red Blood Cells in Umbilical Cord Blood as A Marker of Neonatal Asphyxia with Meconium Stained Amniotic Fluid

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ABSTRACT:

BACKGROUND:

Neonatal asphyxia is a major cause of neurologic morbidity and mortality. Recent studies suggest increased nucleated red blood cells in neonates with meconium aspiration syndrome, supporting that the pregnancy with meconium stained amniotic fluid is at high risk of fetal hypoxia.

OBJECTIVE:

To evaluate the role of umbilical cord nucleated red blood cells as a marker of neonatal asphyxia with meconium stained amniotic fluid.

PATIENTS AND METHODS:

This study was conducted at the Department of Obstetrics and Gynecology and Nursery Department in AL- Kadhymia Teaching Hospital from April 2010 through April 2011as prospective case controlled study. The study included one hundred pregnant women, who were admitted to the delivery ward, they were arranged into two groups. Group one included fifty women with meconium stained amniotic fluid, considered as study group and the other group included fifty women with clear amniotic fluid, considered as control group. This for determination of umbilical cord nucleated red blood cells and comparison between two groups.

RESULTS:

The percentage of abnormal nucleated red blood cells count (NRBCs) in the study group (30%) was significantly more than the control group (18%) (P value =0.002).The mean nucleated red blood cells count (NRBCs) difference was 3.69 units and it was significantly higher in the study group than the control group (P value =0.0002).

CONCLUSION:

The nucleated red blood cells (NRBCs) count increase in cord blood of neonates with meconium stained amniotic fluid compared to neonates of clear amniotic fluid.

KEYWORDS: nucleated red blood cells (nrbc), meconium stained amniotic fluid (msaf), neonatal asphyxia.

INTRODUCTION:

Amniotic fluid surrounds the fetus in intra uterine life, providing protected, low resistance space suitable for growth and development [1]. Clinical assessment of amniotic fluid volume is unreliable. It is more reliable to be measured by ultrasound techniques [1,2,3,4,5]. Meconium stained amniotic fluid composed of swallowed amniotic fluid debris, bile pigments and residue from intestinal secretions, excretions and desquamation of gastrointestinal tract of the fetus. The dark green black colour of meconium is due to pigments mainly biliverdin. Normally it is present in the fetal colon near term and passed within the first 36 hours after birth[6,7].

Asphyxia is a term used to identify infants who have hypoxia (low blood and tissue O_2) and metabolic acidosis (high H^+ concentration in blood and tissues).It may be a cause of neurological damage of newborn (neonatal encephalopathy and cerebral palsy) . Commonly asphyxiated babies end either with death or living with intact brain .In human fetus, elevated plasma erythropoietin level has been found in

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high risk pregnancies at delivery. As hypoxaemia is the most important stimulus of erythropoietin synthesis both in adults and in fetuses, and because erythropoietin does not cross placenta, chronic intra uterine hypoxaemia associated with intra uterine growth restriction is probably responsible for the elevated erythropoietin level in umbilical cord plasma[8, 9].

Although nucleated red blood cells (NRBCs) are rarely found circulating in old children's blood, they are commonly seen in blood of newborn, primarily produced in fetal bone marrow in response to erythropoietin & stored in marrow as precursors to reticulocytes and mature erythrocytes [9].The precise mechanism of increase in NRBCs in hypoxia is unknown (especially for acute onset) , but may be attributed to increase level of cord blood erythropoietin within 1 to 4 hours following acute hypoxia , promoting release of NRBCs from bone marrow stores as high titers of erythropoietin accelerates mitotic division of normoblast from bone marrow. Increase bone marrow blood flow and increase porous infrastructure of the marrow causing relatively rigid large normoblast to escape to circulation (rapid release after acute hypoxia). The precise time to observe increase in NRBCs after hypoxia is not known, but it may be less than 60 minutes & perhaps as short as 20_30 minutes. While chronic hypoxia causing tissue hypoxia that leads to increase release of cord blood erythropoietin & increase release of NRBCs [9, 10, 11, 12, 13].

AIM OF THE STUDY:

To evaluate the role of umbilical cord nucleated red blood cells as a marker of neonatal asphyxia with meconium stained amniotic fluid.

PATIENTS AND METHODS:

This prospective case controlled study was conducted at the department of obstetrics and gynaecology with the nursery care unit at Al-Kadhymia Teaching Hospital for a period of one year (April 2010 to April 2011). The study was approved by Iraqi Scientific Council of Obstetrics and Gynaecology. An informed consent had been taken from the parents of the newborns before collection of umbilical cord blood samples of their newborns. This study included 100 pregnant women, who were delivered at that time; they were divided in to 2 groups:

Study group *(Group A):* Included 50 women who delivered newborns with meconium stained amniotic fluid.

Control group *(Group B):* Included 50 women who delivered newborns with clear amniotic fluid.

For both groups, gestational ages were calculated depending on their regular last menstrual cycles and or their early pregnancy ultrasound. Intermittent fetal heart monitoring was done for both study and control groups and paediatric assessment of the newborns was done. The newborns with meconium stained amniotic fluid MSAF received by paediatrician and were subjected for airways clearing, also birth weights and APGAR scores (1 and 5 minutes) of the studied newborns were assessed.

In an attempt to control various variables known to affect NRBCs counts, exclusion criteria included any woman with chorioamnioniti, previous history of low birth weight infants, Rh-incompatibility, ante partum haemorrhage, Diabetes mellitus, essential hypertension and Preeclampsia. Infants with low birth weight, cyanotic heart diseases, signs of fetal distress, chromosomal anomalies and perinatal blood loss were excluded.

After delivery of the newborns, the umbilical cord was double clamped; then 2 ml of umbilical cord blood was obtained using a syringe into a test tube containing anticoagulant material ethelene diamine tetraacetic acid (EDTA).A complete blood count was performed, blood smear stained with Leishman´s stain was prepared and number of NRBCs per 100 white blood cells was determined (which is normally less than 10 NRBCs/100 WBCs).NRBCs were expressed as a percentage of WBCs per cubic millimeters. All the results had been read by the same pathologist unit at Al-Kadhymia Teaching Hospital.

Regarding statistical analysis; data on the characteristics of patients and the NRBCs count of umbilical cord of newborns with clear and meconium stained amniotic fluid were gathered and analyzed using Smith´s statistical package for Social Science soft ware (version 2.80).The student's *t*- test, Fischer´s exact test were used to compare variables between the 2 groups. Spearman´s correlation coefficient was used to investigate the relationship between NRBCs counts of umbilical cord of newborns with clear and meconium stained amniotic fluid.

A *p*-value of less than 0.05 was considered statistically significant.

RESULTS:

Table 1: Shows patients characteristics; Mean maternal age, gestational age and parity in study &control groups. There was no significant difference in mean maternal age and gestational age between the study group & the control group.

Table 1: **Patient characteristics in the study and control groups.**

Maternal age (years)	25.4200±7.028	28.3529±5.8543	28.4600±8.0539	0.8
Gestational age (weeks)	38.1500±1.8769	38.0196±1.9338	38.2600±1.8161	0.6
Parity	4.2700±3.0743	3.9804±3.0364	4.6400±3.1217	0.2

Table (2) Shows that in the Study and Control groups mean nucleated red blood cells count NRBCs/ 100WBC were 8.2600 ± 1.9487 and 5.6600 ± 2.6696 respectively. In comparison between the study group and control group it had been found that the mean NRBCs difference was 3.69 units and it was significantly higher in the study group than the control group (P value =0.0002). In the study and control groups the percentage of abnormal NRBCs were (30%) and (18%) respectively, it was significantly higher in the study group (P value =0.002).

Table 2: Percentage of nucleated red blood cells count NRBCs in the study and control groups.

	All subjects n=100	Study group n=50 A	Control group n=50 B	*P* value
NRBCs/mm^3	752±410.0948	1.022±152.9172	634±174.5081	0.002
NRBCs/100WBCs	6.9700±2.6722	8.2600±1.9487	5.6600±2.6696	0.0002
NRBCs% Normal NRBCs (<10) Abnormal NRBCs (≥10)	 75% 25%	 70% 30%	 82% 18%	 0.002

Table (3) shows comparison of birth weight and APGAR scores of study and control groups. There was no significant difference in the birth weight of study and control groups .The mean 1 minute APGAR scores of neonates in the study group range between (5-7) which was significantly lower than the mean 1 minute APGAR scores of neonates in the control group with range between (7-9), (*p* value = 0.02).There was an inverse relationship between NRBCs count and APGAR scores of 1 and 5 minutes.

Table 3: Comparison of birth weight andAPGAR scores of neonates at 1 and 5minutes of study and control groups.

	All subjects n=100	Study group n=50 A	Control group n=50 B	*P* value
Birth weight APGAR (1 min s)	3.154±609.4621 7.8600±1.524	3.055±595.0786 7.020±1.5842	3.270±6137872 8.4500±1.0539	0.06 0.003
APGAR (5 min s)	8.4500±0.9679	8.3600±0.8751	8.4500±1.0539	0.003

Figure (1) Shows relation of NRBCS count /100 WBCs to APGAR scores of 5 minutes and severity of acute asphyxia, showing that neonates with low 5 minutes APGAR scores have higher NRBCs counts, showing the importance of NRBCs as early detector of neonatal asphyxia.

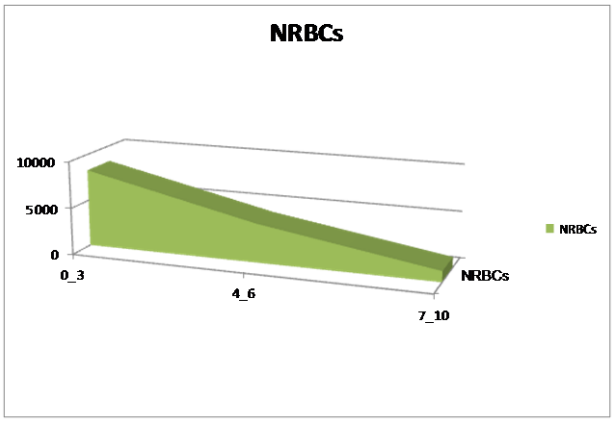

Figure 1: Relation between NRBCs & APGAR scores of 5 minutes of newborns with MSAF, results are meaning (SD).

DISCUSSION:

Passage of meconium stained amniotic fluid is considered as a sign of fetal hypoxia (distress) over short or long period of time, and as it is present in about 10% of term pregnancies (quite rare in pre term) [13].This hypoxic status & decrease in oxygen tension induces a fetal compensatory response in the form of exaggerated erythropoiesis as a result of increase erythropoietin level leading to influx of immature red blood cells (NRBCs) into the fetal circulation which may be correlated with presence of perinatal asphyxia [9,13].

According to our study there is significant increase in cord blood NRBCs/100 WBCs in the neonates of MSAF compared with those of clear amniotic fluid.

Esmaeilian L. & Jahanfar M. agree with our results, they stated that the mean NRBC counts in chronic fetal distress group were higher than acute fetal distress. NRBC counts were found to be correlated with umbilical cord pH (r=−0.57; P<0.001). The cutoff value predicting fetal acidosis was determined as 14/100 leukocytes (sensitivity 87%, specificity 81%) by using ROC analysis. NRBCs count in his study group was (12.04/ 100WBCs), compared with (3.75/100 WBCs) in the control group with mean difference of (8.29 units), and also showed increase in abnormal NRBCs in the study group (32 %), compared with (9%) in the control group[14].

Torkstani F. et al. results support ours in that they found the number of NRBCs in neonates of MSAF was (9.98 cell/ 100 WBCs) which is high compared with (8.19/WBCs) in the control group [15].

Dasari Pappa et al. agree with our results in that the mean NRBCs count in the study group was high (25.67/100WBCs) as compared to (12.33/ 100 WBCs) in the control group with mean

difference of (12.32 units) [16].

Hassan Boskabadi et al. also have similar results to our study &the NRBC/100 WBC and absolute nucleated red blood cell levels in the blood of newborns in the control group were 3.87±5.06 and 58.21±87.57/mm^3, respectively; whereas the corresponding values in the case group were 18.63±16.63and 634.04±1002/mm^3, respectively (P<0.001). A statistically significant negative correlation existed between nucleated red blood cell level and indicators of the severity of perinatal asphyxia, first minute APGAR score and blood pH (P<0.001),respectively. A positive correlation was demonstrated between these parameters and severity of asphyxia, acidosis, and poor outcome (P<0.05) [17].

In our study we also found that there was difference between 1 and 5 minutes APGAR scores of neonates with MSAF (range between 5- 7) when compared to those of control group (range between 7- 9), also we found an inverse relationship of APGAR scores (of these neonates) with their cord blood NRBCs count. That was also agreed by Dasari Pappa et al. [17] who found significantly high NRBCs counts in neonates with MSAF with APGAR scores ≤ 5to 6.

Hassan Boskabadi et al. [18] stated a significant negative correlation between NRBCs & indicators of severity of asphyxia in neonates including 1 minute APGAR score.

According to Torkstani F. et al [16] study, they found no positive relationship between NRBCs and 1 or 5 minutes APGAR scores.

Saracoglu F. et al. demonstrated that the mean NRBC counts in chronic fetal distress group was higher than in those with acute fetal distress and concluded that the duration and the severity of fetal asphyxia may be predicted by the number of NRBCs per leukocyte [18].

Through our study we found that there was no significant difference in gestational age of the study group & that of the control group, But NRBCs have an inverse relation with the gestational age [9].

CONCLUSION:

The NRBCs/WBCS counts increase in cord blood of neonates with MSAF compared to neonates of clear amniotic fluid and we found inverse relationship between NRBCs and gestational age of the neonates and the inverse relationship between NRBCs count and APGAR scores at both 1 and 5 minutes.

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