Nucleated Red Blood Cells in Cord Blood of Singleton, Term Neonates and The Risk Factors Affecting Their Count

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Summary:

Background: *this study aims to establish normal values for nucleated red blood cells (NRBCs) in healthy, term, singleton neonates and to evaluate the association between some medical and obstetrical conditions, known to be associated with chronic hypoxia, and NRBC count elevation.*

Setting: *we prospectively collected umbilical cord blood from 349 live born, term, singletons, delivered at our department. Cord blood was analyzed for nucleated RBC. We reviewed the medical record for maternal data and neonatal outcomes of gestations of >. 37weeks duration .The cases were divided into two main groups on the basis of the presence or absence of complicated pregnancies.*

Results: *The mean value for NRBCs per 100 WBCs in control group (those with uncomplicated pregnancies) was 2.4,the SD was 2.1, and the range was 0 to 10. There was significant elevation of NRBCs in cases of small for gestational age. and in cases complicated by maternal diabetes or pre-eclampsia (p< 0.05).The value did not vary by maternal smoking.*

Apgar score showed trend toward inverse proportionality to the number of NRBCs.

Conclusion: *the NRBCs counts in the healthy, term neonates were in the range of 0-10. Elevated NRBC count was associated with states of relative hypoxia such as intrauterine growth restriction, maternal diabetes and preeclampsia. Elevated NRBC count were also associated with lower Apgar scores.*

Keywords: *Nucleated red blood cells, fetal hypoxia, intrauterine growth restrictions, gestational diabetes, pregnancy induced hypertension.*

Introduction:

Nucleated red blood cells (NRBCs) are immature erythroblasts that were first noted in 1871 to be present in the blood of neonates [11]. Until the sixth and seventh weeks of gestation, particularly all fetal red blood cells are nucleated. By the twelfth week of gestation NRBCs count decline and are uncommon in the circulation of term newborns[1]Their incidence and significance at term has been a matter of controversy.

The number of NRBCs per 100 WBCs is quite variable but is rarely more than 10 t2). In these instances in which the number of NRBCs exceeds 10 ,the most frequent explanations for increase are prematurity and rhesus sensitization (2) .while the presence of increased number of NRBCs in the circulation of term infants has been associated with states of relative hypoxia , such as intrauterine growth restriction , maternal diabetes and preeclampsia13'. NRBCs have also been implicated as a possible marker of perinatel brain damage (3)

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The heamopoitic system respond to hypoxia by increasing Erythropoietin (EP) which increases erythroid production, and releasing less mature forms into the circulation [M]. The only known stimulus for (EP) is tissue hypoxia, which has been well documented in both human fetuses and animal models [56], and since (EP)does not cross the placenta [7] ', increased fetal plasma levels of (EP) are indications of fetal hypoxemia[7 8]. Since there is a significant association between fetal (EP) and NRBCs count[479], assessment of NRBCs in cord blood regarded as simple and cheep method of assessment of intrauterine fetal life , and this may have important implication in determining causality in cases of compromised infants .

This study aims to highlight the normal range of NRBCs count in a sample of Iraqi ,healthy, term singleton neonates, and to confirm the relation of certain Medical and Obstetrical conditions, known to cause chronic fetal hypoxia , to the changes in NRBCs count .and to evaluate NRBCs as a new marker in the detection of chronic intrauterine hypoxia.

PATIENTS & METHODS

We prospectively studied 349 pregnant women delivered at the department of Obstetrics & Gynecology - Baghdad teaching hospital between June 1st 1998 and Oct 31st 1999. All cases were of singleton, term pregnancies (gestational age >. 37

and <_ 42 weeks) by last menstrual period and / or early ultrasound.

Immediately after delivery blood was drown from the umbilical vein into an ethylenediamine tetraacetic acid (EDTA) tube for hematological analysis. Samples were labeled (NRBC study) and sent to the hospital laboratory, in the lab a slide was prepared with Leishman stain and NRBCs were manually counted by trained hospital lab technicians. A complete blood cells count was performed, the total WBC count was initially determined, the number of NRBCs was determined by an examination of blood smear from the differential WBC count. For purpose of this study the number of NRBC per 100 WBC is expressed as the NRBCs count. The neonatologist weighed, examined the babies and the Apgar score was assessed in one and five minutes, the reference range used for Apgar score was 7 to 10. W[1]e classified patients into two main groups according to the following criteria; Group one (control group) consisted of 200 infants delivered to healthy mothers with no history of obstetrical or medical complications or smoking during pregnancy, normal intrapartum fetal heart pattern, clear amniotic fluid, their birth weight appropriate for gestational age (>2500gm), Apgar score of > 7 at 1 and 5 minutes ,and normal neurological evaluation at discharge. The second group consisted 149 infants delivered to mothers with potential risk factors for hypoxia, these include diabetes , pregnancy induced hypertension (PIH), small for gestational age (SGA) , maternal smoking . Maternal diabetes include both pregestational and gestational on insulin treatment. Pregnancy induced hypertension (PIH) were defined as maternal blood pressure in the third trimester either diastolic BP^_ 110 mmHg, or Diastolic BP> 90 mm Hg on two occasions four hours apart, with or without protein- urea with normal BP reading in the first half of pregnancy according to their antenatal records" . Small for gestational age (SGA) include term infants weighing less than 2500 gm or below the tenth percentile for gestational age on Brunner curves[11], hypertensive and smoking mothers were excluded from this group. Maternal tobacco smoking was determined by self-identification in prenatal care or at presentation for deliver}'. The ANOVA test with LSD procedure was applied to study changes in mean NRBCs count in the different groups of the study, the regression analysis was used to show the linear relationship between Apgar score and NRBCs count. The difference was considered to be statistically significant when the P value was less than 0.05.

RESULTS:

A total number of 349 live borne, terms, singleton neonates who delivered at our department, were studied, hi 20 cases the cord blood specimen was unacceptable for technical reasons (e.g., insufficient quantity of specimen or specimen clotted), leaving 329 cases in which NRBCs are studied. Demographic data for the stud}' population are shown in (able 1 .The mean gestational age of our study population was (38.2 ± 1.5) weeks, with a mean birth weight of (3228 ± 416)gm .the mean maternal age was (22.3 ± 5.6) years .with 23.4 % primiparus women . The mean value of NRBCs per 100 WBC in the control group (n=200) was 2.4 ,SD2.1, range 0-10.

Table (1) : Maternal demographic, gestational age and birth weight data.

	Number	Mean	SD	%
Gestational age (wk)	**329**	**38.2**	**1.5**	
Birth weight (gm)	**329**	**3228**	**416**	
Maternal age (yr)	**329**	**22.3**	**5.6**	
Gravidity				
1	**77**			**23.40**
2	**85**			**25.83**
3	**93**			**28.26**
4	**74**			**22.49**

Figure (1) displays graphically the concentration of control group cases with lower values and the long, gradual spread of the data as the values of NRBCs per 100 WBC increased.

The second group comprise 129 cases which includes antepartum factors in relation to counts of NRBCs per 100 WBC and divided into cases of maternal diabetes (n=32), maternal pregnancy induced hypertension (n=45), cases of small for gestational age (n=32), and maternal smoking (n=20).

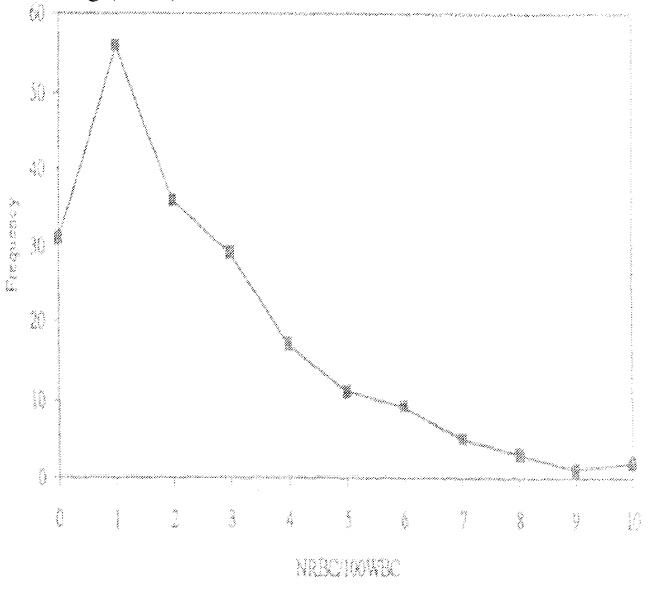

Figure (1): Distribution of Nucleated red blood cells (NRBC) per 100 whiteblood cells (WBC) in group one.

Table (2) and figure (2) shows the relation of NRBCs count in the second group as compared to control group .We found that there is statistically significant higher counts in cases complicated by maternal diabetes, pregnancy induced hypertension , and small for gestational age as compared to control group,(mean 23.15 ,SD 8.79 ,mean 17.84 SD5.93 mean 46.68 SD 7.76 and mean 2.40 SD 2.12) respectively, while there is no statistical difference in counts was found for maternal smoking compared to control group (mean 2.70 ,SD 2.22 and mean 2.40 ,SD2.12) respectively p> 0.6

Table (2) ; the relation of NRBCs count in the second group as compared to control group

	No.	Mean NRBCs	SD	Standard error	minimum	maximum
Control	200	2.400	2.124	0.150	0.00	10.00
Diabetes	32	23.156*	8.795	1.554	6.00	36.00
PIH	45	17.844*	5.938	0.885	6.00	32.00
JiGA	32	46.687*	7.768	1.373	36.00	70.00
Smoking	20	2.700#	2.226	0.497	0.00	7.00

*P<0.05
P>0.6

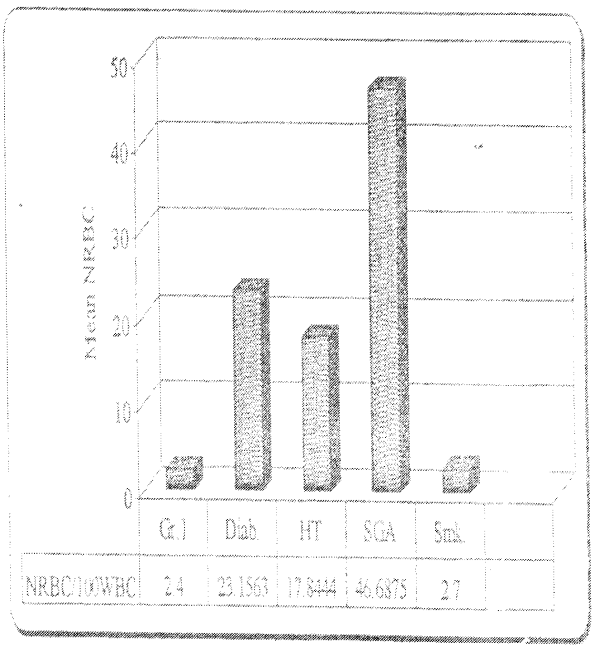

Figure (2): The mean NRBC counts in the whole groups of the study.

Table (3) : Apgar scores and NRBCs counts per 100 WBCs

Apgar score	Number	Mean NRBC/WBC	SD	Range
0-3	3	46.00 **	20.880	32.00-70.00
4-6	12	38.416 *	13.905	25.00-60.00
7-10	314	8.486	12.510	0.00-53.00
0-3	1	70.00	.....	70.00
4-6	5	44.40 *	13.870	32.00-60.00
7-10	323	9.04	13.07	0.00-54.00

- * P< 0.05 compared with Apgar scores of 7-10
- ** P< 0.01 compared with Apgar scores of 7-10

Apgar score at one and five are shown in table (3). NRBCs per 100 WBC tended to be inversely proportional to the Apgar score at both 1 and 5 minutes. One minute Apgar score of 0 to 3 was significantly associated with higher counts than were Apgar scores of 7 to 10.

DISCUSSION:

In tills prospective study we set out to establish normative data for NRBCs in cord blood of healthy, singleton, term neonates and to evaluate the association between some Medical and obstetrical conditions known to cause chronic hypoxia, and NRBCs elevation.

Our results show NRBCs counts in the non asphyxiated term neonates in the range of 0-10 and are in keeping with those observations that found NRBCs to be less than 10 per 100 WBC in the blood of healthy term newborn infants. [2-4]

In normal human life the number of circulating erythroblasts decrease exponentially with gestation [3-5] . This decrease coincides with the switch from hepatic to medullary erythropoiesis, with liver erythropoiesis, erythroblasts enter the peripheral circulation freely, whereas with marrow erythropoiesis the nucleated erythroid precursors are confined to the parenchyma in which hematopoiesis takes place[9131].

Our subgroup of diabetic mothers, showed a significant elevation of infants cord blood NRBCs compared to control group. Our finding was compatible with the data of previous studies. In their studies. Green et al 1990 [(1)], and Hanlon-Lundberg 1997 [-11] found a higher NRBCs count in

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Cases with PIH was associated with significantly increased NRBCs , these findings were in accordance with previous studies that demonstrated elevated NRBCs count in cases complicated by preeclampsia [1)]. Intrauterine hypoxia has been proposed as the common underlying metabolic stimulus for increased erythropoiesis in PIH [nSI]. In PIH deficient implantation results in a reduction of maternal - placental blood flow [oSj].

In this study of 32 small for gestational age (SGA) infants, there was a significantly increased NRBC in the cord blood at delivery . this result is in agreement with previous studies, which found that the NRBCs count of infants who were SGA were considerably higher at birth[31:c:n].

Growth retarded fetuses are believed to be at significantly elevated risk for adverse perinatal out come as a result of abnormal gas exchange and decrease transport of nutrient across the maternal placental interface [0V]. In SGA fetuses at least two factors contribute to tissue hypoxia, first there is hypoxemic hypoxia (low blood oxygen contents), caused by reduced uteroplacental perfusion and oxygen transport to the fetus , second is ischemic hypoxia which occurs as a result of redistribution in fetal blood flow with preferential shunt to the brain, heart and adrenals '.

The positive correlation between hypoxia and NRBC count indicate an erythropoitic response in these fetus which would tend to maintain blood oxygen content[20)].

In this study of 20 smoking mothers there was no significant elevation of NRBCs when compared to control group, probably because the size of the sample was small , or because of the difficult quantification of the actual number of cigarette

smoked per day during the entire pregnancy. This finding is inconsistent with other studies which find significantly elevated number of NRBCs and EP in cord blood of infants of smoking mothers'[2223]). There are several reasons why cigarette smoking is likely to effect fetal oxygenation. The first is that nicotine is a potent vasoconstrictor and any interference with the caliber of the placental vessels will reduce the blood flow through this organ. The second reason is that inhalation of carbon monoxide in the concentration found in cigarette smoke results in a fall in arterial oxygen tension

Apgar score was proposed as a method of quickly evaluating the status of the neonates([24]) . Hypoxia may be associated with depressed Apgar scores as well as with stimulation of (EP)- NRBCs production. Our data support an inverse relationship between NRBCs and Apgar scores.

Conclusion

In this limited population, NRBCs count appears to aid in identifying the presence of fetal Hypoxia and opens the possibility for the development of a clinical tool capable of determining chronic Hypoxia at birth. The NRBC test is easy to apply since the cord blood may be obtained non-invasively from an otherwise discarded specimen and analyzed by personal on equipment readily available in most hospital laboratories.

This test can be used prenatally by means of cordocentesis. Although it is an invasive way of blood sampling, but it can detect which fetus is really experiencing intrauterine Hypoxia and might help delivery to be timed to achieve the best postnatal prognosis.

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