

Artigo / Article

Erythrocyte indices and serum ferritin in newborns

Índices de eritrócitos e ferritina sérica em recém-nascidos

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Hematological values for newborn babies differ depending on the gestational age and intrauterine growth. However, information in published studies about hematological values for cord blood according to weight-for-gestational-age categories is limited and inconsistent, especially regarding small-for-gestational-age (SGA) newborns. With the aim of describing hematological values for umbilical cord blood, we conducted a cross-sectional study in two government maternity hospitals in Salvador, Brazil. Eighty-eight healthy, term, appropriate-for-gestational-age (AGA), 23 term, small-for-gestational-age (SGA), and 13 preterm, AGA newborns were studied. Means and standard deviation, and maximum and minimum values were used to describe the level of hemoglobin (Hb), red blood cells (RBC), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red cell distribution width (RDW) and serum ferritin. The highest values of RBC, Hb, HCT and serum ferritin were recorded for term SGA newborns and the lowest values were recorded for preterm AGA newborns. Term AGA newborns had intermediate values for all these parameters. In this study we observed the highest hematological values in SGA newborns. Because, these infants are at the highest risk of iron deficiency, the use of more detailed screening tests for iron deficiency in these newborns at birth, using a combination of biomarkers, should be considered.

Keywords: Infant; Small for gestational age; Fetal blood; Erythrocyte indices; Ferritins

Introduction

At birth, hematological values are higher when compared to those observed in older children or adults. This is in response to increased erythropoiesis caused by intrauterine hypoxia.⁽¹⁾ In addition, hematological values in newborns vary according to gestational age and fetal growth. Some investigators have reported term, small-for-gestational-age (SGA) newborns as having higher levels of hemoglobin (Hb), red blood cells (RBC) and hematocrit (HCT), compared to appropriate-for-gestational-age (AGA) newborns.⁽²⁻⁵⁾

Furthermore, studies have reported lower values for preterm AGA newborns.^(3,6)

Reference hematological values for cord blood have not been standardized, and routinely out of date values proposed in the literature are used.⁽⁷⁾ In published studies, information on hematological values in cord blood is limited and inconsistent. The studies that we found^(3-6,8-10) evaluated different subgroups of neonates and different hematological parameters. In particular, little data regarding hematological parameters of SGA newborns was found.^(3-5,9) However, the impaired fetal growth and/or the condition of SGA are

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associated with increased infant mortality and various morbidities, including hematological changes.

Hence, the objective of this study was to describe the hematological profile of umbilical cord blood and iron reserve in three distinct groups of newborn babies: term AGA, term SGA and the preterm AGA; and to compare these results with those reported by other authors.

Methods

This is a descriptive, cross-sectional study of 124 newborns at two government maternity hospitals in Salvador, Bahia, Brazil. Newborns were prospectively and consecutively enrolled between June 2005 and October 2006. We included all newborns delivered vaginally or by cesarean, born at ≥ 30 and ≤ 41 weeks of gestation. They were divided into three groups: Group 1 (the controls): eighty-eight healthy, term (gestational age ≥ 37 and ≤ 41 weeks), AGA newborns (birth weight ≥ 10 th and < 90 th percentile). Group 2: twenty-three term, SGA newborns. Group 3: thirteen preterm (birth weight < 37 weeks), AGA newborns. Small-for-gestational-age was defined as birth weight < 10 th percentile for gestational age.⁽¹¹⁾

Newborns were excluded if they were large for gestational age (birth weight ≥ 90 th), were multiple births or had chromosomal anomalies, intrapartum complications or hemoglobinopathies. In addition, newborns with low Apgar scores (≤ 7) within the first five minutes and those whose mothers had Rh-negative blood were excluded from the control group.

This study was approved by the Ethics Committee of the "Instituto de Saúde Coletiva – Universidade Federal da Bahia" (Institute of Community Health – Bahia Federal University). Informed written consent was obtained from all mothers and all interviews were carried out post delivery, with mothers in good clinical conditions. Mothers with SGA newborns were notified and counseled about the need for further clinical evaluations.

Birth weight was collected from medical records. Gestational age was defined in full completed weeks¹² and measured by the three following methods, according to the best antenatal data provided:

Last Menstrual Period Method (LMP) – This was the primary method for defining gestational age, if the LMP was known and menstrual cycle was regular (25-31 days).⁽¹²⁾ This information was obtained from medical records and confirmed by interview.

Ultrasound Method – Given the potential problems of the LMP method (maternal recall and/or early, non-menstrual bleeding, wrongly interpreted as a period),⁽¹²⁻¹⁴⁾ we used the ultrasound method when an accurate LMP was unknown or the menstrual cycle was irregular. Only early ultrasound measurement (< 10 weeks) of the crown-rump length or (< 20 weeks) of the biparietal diameter or femur

(diaphysis) length were used. All of them are considered good standards.^(13,15) This information was obtained from medical records. If the record could not be found, we requested a copy of test results directly from the patient.

Capurro Method (physical) – In the absence of information about LMP and ultrasound, the Capurro method⁽¹⁶⁾ was used, regardless of its limitations.⁽¹⁷⁻¹⁹⁾ This information was obtained from medical records. When the information from the three previously noted methods was unavailable, the Capurro method was performed by trained interviewers. The interviewers evaluated 4 newborns.

Blood Sampling – Blood samples were collected from the umbilical cord. In all cases the placental cord was clamped immediately after delivery. Two blood samples, consisting of 5 ml each, were collected. The blood samples were kept refrigerated and sent immediately for processing. One sample with ethylenediaminetetraacetic acid (EDTA) was sent for complete blood count and for diagnosis of hemoglobinopathies. The second sample was used to evaluate the serum ferritin.

Hematological Analyses were performed with a Pentra Retic 80 Horiba/ABX coulter analyzer. The following hematologic parameters were determined: hemoglobin (Hb), red blood cells (RBC), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and red cell distribution width (RDW).

Ferritin was measured by the radioimmunoassay (RIA) technique (IMIX Ferritin) using Access Immunoassay Systems (Imunosystems) equipment from Abbot Laboratories-USA.

Evaluation of hemoglobinopathies was carried out by High Performance Liquid Chromatography (HPLC), by β -Thalassemia Short Program on the Bio-Rad Variant, HPLC Analyzer System (Bio-Oxford Ltd).

Maternal Characteristics – The following maternal data were collected through standardized pre-coded questionnaires, that were completed in face-to-face interviews: Socio-economic Status – was defined according to the Criteria of the Economic Classification in Brazil (Critério de Classificação Econômica Brasil – CCEB)⁽²⁰⁾ as proposed by the Brazilian Association of Survey Companies (Associação Brasileira de Empresas de Pesquisa – ABEP). According to these criteria, seven categories were established: Groups A1 and A2 (high), Groups B1 and B2 (intermediate), Group C (intermediate-low), Group D (low) and Group E (very low). Given the small numbers in groups A and B, and for analysis purposes, it was decided to group these categories into group C. Therefore, the Groups A, B, and C were renamed Group C (reference category).

Color/race – was classified in accordance with the categories of white, brown (*mulatto*), black, yellow, and indigenous. These categories were proposed by the Brazilian Institute for Geography and Statistics (Instituto Brasileiro de Geografia e Estatística – IBGE)⁽²¹⁾ based on self-classification.

The interviewee had to answer the question, "What is your color or race?" and was asked to choose one of the above mentioned options. Due to the small number of samples in the categories of "yellow" and "indigenous," these two categories were placed into the existing "*mulatto*" category. These three categories, *mulatto*, yellow and indigenous, were then renamed as "others." White color/race was adopted as the reference category in order to compare against the categories of "black" and "others." In addition, maternal information on age, smoking, and method of delivery were collected.

Statistical analysis

Descriptive analysis was carried out using the statistical software package SPSS, version 11.0 (SPSS Inc, Chicago, IL). The study used means and standard deviation and both maximum and minimum values to describe the hematologic parameters.

Results

Sample characteristics

All newborns tested for hemoglobinopathies were normal and therefore none was excluded for this condition. Out of 124 newborns selected for this study, 46% were male. The mean gestational age for term newborns was $39.42 \pm$

1.21 weeks, and for preterm babies was 34.69 ± 2.29 weeks. Mean birth weights were 3290.55 ± 300.52 g for term, AGA newborns; 2583.26 ± 271.99 g for term, SGA newborns and 2533.08 ± 754.95 g for preterm, AGA newborns (data not shown). Table 1 presents some of the selected maternal characteristics. According to this table, most of the mothers classified themselves as black (44.4%). The socioeconomic status was mainly intermediate (50.0%) and low (46.0%). Most of them had not smoked (90.3%), and had taken iron supplements during pregnancy (72.6%).

Capurro method

We evaluated the reliability of the Capurro method performed by interviewers. The intraclass correlation coefficient (ICC) was calculated in five percent of the sample, and there was no evidence of inter-observer variability ($\rho=0$)⁽²²⁾ (data not shown).

Hematologic parameters

Hematologic parameters of umbilical cord blood are presented in Table 2. Mean values for RBC were higher in term, SGA newborns ($4.46 \times 10^{12}/l \pm 0.49$); slightly lower in term AGA ($4.21 \times 10^{12}/l \pm 0.48$); and even lower in preterm AGA ($4.00 \times 10^{12}/l \pm 0.52$). Similar patterns were observed for Hb and Hct. Term SGA newborns presented the highest mean values, with Hb at 15.07 g/dl ± 1.26 and Hct at $45.33\% \pm 3.73$. Preterm AGA newborns presented the lowest values, with Hb at 14.26 g/dl ± 1.94 and Hct at $42.56\% \pm 5.78$.

MCV and MCH exhibited a different pattern. Preterm AGA newborns showed the highest mean values, 106.30 fl ± 3.52 and 35.53 pg ± 1.38 , respectively. Term SGA newborns showed the lowest mean values, with MCV at 102.08 fl ± 7.08 and MCH at 33.91 pg ± 2.69 .

The mean values of RDW were similar in the different groups of newborns (Table 2). Term SGA newborns presented a slightly higher mean value ($13.56\% \pm 0.70$) than term AGA and preterm AGA newborns ($13.33\% \pm 0.85$ and $13.38\% \pm 0.86$, respectively). Term SGA newborns also presented the highest mean values for serum ferritin (190.89 μ g/l ± 144.79) and preterm AGA newborns presented the lowest (142.23 μ g/l ± 65.55).

Discussion

In this study, we observed that term, SGA newborns presented the highest mean values for Hb, RBC, Hct and ferritin and preterm, AGA newborns exhibited the lowest mean values for these parameters. Preterm, AGA newborns however, presented the highest mean values for MCV and MCH. Term AGA newborns showed intermediate values for all these parameters (Table 2). Higher mean values for Hb, RBC and Hct, in term SGA newborns compared to other newborns, have previously been reported in other studies.⁽³⁻⁵⁾ It is known that chronic fetal hypoxia, due to poor placental function in

Table 1. Maternal characteristics according to the newborn babies' weight-for-gestational-age-category at two government maternity hospitals in Salvador

Maternal characteristics	Newborns			Total %
	Term AGA n (%)	Term SGA n (%)	Preterm AGA n (%)	
Color/Race ^a				
White	13 (14.8)	3 (13.1)	2 (15.4)	14.5
Black	39 (44.3)	11 (47.8)	4 (30.8)	44.4
Other	36 (40.9)	9 (39.1)	7 (53.8)	41.1
Age (years)				
<20	12 (13.6)	4 (17.4)	2 (15.4)	14.6
20 - 35	66 (75.0)	18 (78.3)	8 (61.5)	74.8
35	10 (11.4)	1 (4.3)	3 (23.1)	10.6
Socioeconomic status ^b				
Intermediate	43 (48.9)	11 (47.8)	8 (61.5)	50.0
Low	43 (48.9)	9 (39.1)	4 (30.8)	46.0
Very low	2 (2.2)	3 (13.1)	1 (7.7)	4.0
Smoker				
No	83 (94.3)	19 (82.6)	10 (76.9)	90.3
Yes	5 (5.7)	4 (17.4)	3 (23.1)	9.7
Method of delivery				
Vaginal	60 (68.2)	19 (82.6)	6 (50.0)	69.1
Cesarean	28 (31.8)	4 (17.4)	7 (50.0)	30.9
Iron supplementation				
Yes	63 (71.6)	17 (73.9)	10 (76.9)	72.6
No	25 (28.4)	6 (26.1)	3 (23.1)	27.4

GAG adequate for gestational age, SGA small for gestational age. ^aIBGE (2001)

^bCCEB; available at http://www.abep.org/codigosguias/ABEP_CCEB_2003.pdf

Table 2. Hematological values of umbilical cord blood according to Newborn babies' weight-for-gestational-age-category at two government maternity hospitals in Salvador

Newborn group*	RBC (x10 ¹² /l)	Hb (g/dL)	HCT (%)	MCV (fl)	MCH (Pg)	RDW (%)	Serum ferritin (mg/L)
Term AGA n=88	4.21 0.48 (3.24-5.89)	14.50 1.42 (11.50-18.30)	43.50 4.36 (33.50-54.40)	103.66 5.30 (89.00-115.00)	34.52 2.11 (30.00-39.00)	13.33 0.85 (11.80-16.20)	162.61 100.10 (14.45-577.90)
Term SGA n=23	4.46 0.49 (3.56-5.35)	15.07 1.26 (12.60-17.50)	45.33 3.73 (37.60-52.60)	102.08 7.08 (84.0-110.00)	33.91 2.69 (27.20-37.00)	13.56 0.70 (12.30-15.30)	190.89 144.79 (55.11-680.40)
Preterm AGA n=13	4.00 0.52 (3.17-4.88)	14.26 1.94 (11.70-17.80)	42.56 5.78 (34.60-53.00)	106.30 3.52 (101.00-113.00)	35.53 1.38 (33.00-38.00)	13.38 0.86 (11.50-14.60)	142.23 65.55 (39.53-240.80)

AGA Adequate for gestational age, SGA small for gestational age, Hb hemoglobin, RBC red blood cells, HCT hematocrit, MCV mean corpuscular volume, MCH mean corpuscular hemoglobin, RDW red cell distribution width. *weight-for-gestational-age. Data are presented as means SD and (range)

SGA newborns with subsequent increases in erythropoiesis, is responsible for this.⁽¹⁴⁾

Our findings of low mean values for Hb, RBC, Hct, and high mean values for MCV observed in preterm AGA newborns, are similar with those reported by other authors.^(3,6) These characteristics of preterm newborns are related to physiologic immaturity of the erythropoietic system.^(23,24)

We evaluated serum ferritin levels at birth as an indicator of iron deposits in newborns. Fetal iron deposit is related to size at birth and gestational length. The average iron content at birth is approximately 75 mg/kg of body weight, with storage occurring mostly during the third trimester of gestation.⁽²⁴⁾ Despite the fact that, infants with intrauterine growth restriction (IUGR) are at risk of iron deficiency,⁽²⁵⁾ term SGA newborns in this study presented the highest mean values for serum ferritin, different to the results of a previous study.⁴ Low serum ferritin values are good indicators of iron deficiency; high values in contrast, are usually associated with inflammation/infection and frequently associated with IUGR.^(24,26)

In fact, serum ferritin may not be the appropriate indicator of iron deposit in SGA newborns; however there is no standard test for the diagnosis of iron status at birth⁽²⁷⁾ and, in this case, a combination of different tests would be appropriate.

Preterm AGA newborns, however, exhibited the lowest mean values of serum ferritin, similar to what was previously reported,⁽⁶⁾ and this is mainly related to their low iron deposit.⁽²⁴⁾

Our study also evaluated RDW. This parameter is a useful tool in the diagnosis of iron status, because it is the first to increase in the presence of iron depletion, followed by the decrease of MCH and MCV. Therefore, these three parameters have been used in the diagnosis of anemia.^(28,29) In this study, mean values of RDW were similar in different groups of newborns, as reported in a previous study.⁽⁵⁾

We compared our findings, in term AGA newborns, to those obtained in others studies that had evaluated hematological parameters in cord blood. Some of them classified newborns according to their weight-for-gestational-

age-categories^(3,5,9) while others did not.^(6-8,10) Our results regarding RBC parameters were similar to those reported in some studies^(3,8,10) and lower than those observed in others.^(5,6,9) We also observed that our results were lower than those usually used as reference standards in neonatology.⁽⁷⁾

We found three probable explanations for the observed differences. One explanation is probably due to the fact that, in this study, we used term AGA newborns as a reference standard instead of term newborns. It is well known that differences exist in hematological parameters of newborns according to their weight-for-gestational-age categories. A second explanation is that, RBC parameters are lower when placental cord is clamped immediately after delivery,⁽³⁰⁾ which was the case in this study. Finally, an additional explanation may be related to the fact that 44.4% of the newborns were from mothers that classified themselves as black. In this population group, the hematological values are lower compared to the other groups.^(31,32)

Comparing our findings regarding serum ferritin levels in term AGA newborns, with those obtained previously, we observed that they were close to the results reported by some authors⁽⁶⁾ and higher than those obtained by others.^(4,10) The mean value for serum ferritin in term AGA newborns in this study (162.61 ± 100.10 µg/l), was in agreement with the literature,²⁵ and it is probably related to the fact that 72.6% of mothers took iron supplements during pregnancy.

Our study presented some limitations. Given the small sample of some groups of newborns, especially the preterm AGA group, we did not perform statistical tests to compare mean values of hematological parameters among different groups of newborns. Another limitation was that we did not perform PCR tests to validate the values of serum ferritin, especially in SGA newborns (190.89 ± 144.79). In addition, there are some limitations specific to each method of gestational age used. However, despite all these limitations, the study shows the hematological parameters in cord blood and identifies the profile of these parameters in SGA newborns, for which the data are limited. We recommend further studies regarding hematological parameters and

screening for iron deficiency in newborns, according to the weight-for-gestational-age categories, using a combination of biomarkers.

Resumo

Valores hematológicos dos recém-nascidos (RN) diferenciam-se em função da idade gestacional e do crescimento intrauterino. Na literatura, as informações sobre valores hematológicos de acordo com categorias de peso para a idade gestacional em RN são escassas e inconsistentes, especialmente referindo-se a RN pequenos para a idade gestacional (PIG). Para descrever os valores hematológicos do cordão umbilical foi realizado um estudo transversal, em duas maternidades públicas de Salvador, Brasil, envolvendo 88 RN a termo adequados para a idade gestacional (AIG), 23 RN a termo PIG e 13 RN pré-termo AIG. A média, desvio-padrão (DP), e valor máximo e mínimo foram usados para descrever os níveis de hemoglobina (Hb), número de hemácias (Hm), hematócrito (HCT), volume globular médio (VGM), hemoglobina globular média (HGM), amplitude de distribuição das hemácias e ferritina sérica. Os valores mais altos de Hm, Hb, HCT e ferritina sérica foram identificados nos RN a termo PIG, e os mais baixos foram encontrados nos RN pré-termo AIG. Os RN a termo AIG apresentaram valores intermediários para todos referidos parâmetros eritrocitários. Neste estudo foram observados valores hematológicos mais elevados em RN a termo PIG, apesar desses RN apresentarem maior probabilidade de deficiência de ferro; indicando a necessidade de um diagnóstico mais detalhado da deficiência de ferro ao nascer, neste grupo de RN, usando a combinação de vários testes.

Descritores: Recém-nascido pequeno para a idade gestacional; Sangue fetal; Índices de eritrócitos; Ferritinas

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