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ORIGINAL ARTICLE

Anemia among premature infants in the first year

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Abstract

Objective: To analyze hemoglobin levels of premature infants in their first year and associated factors. **Methods:** A cross-sectional study of preterm follow-up data at a reference center (n = 93) between September 2010 and December 2015. Outcome: hemoglobin levels in the first year. Descriptive analysis, comparison testes and multiple linear regression were performed to estimate the influence of socioeconomic, perinatal and ambulatory follow-up on hemoglobin levels. **Results:** Low maternal schooling was associated with lower levels of hemoglobin, contributing with 8.7% of its variation. Anemia occurred in 25.8% of premature infants, with a median hemoglobin of 10.2g/dL (9.4-10.6). 37.8% of mothers had low schooling. 82.8% of the preterm infants were breastfed at the first consultation, mean median duration was 5 months. 64.6% of premature infants used cow's milk in natura in the first year. **Conclusions:** Lower hemoglobin levels among preemies were associated in part with lower maternal schooling. The prevalence of anemia among preterm infants was relevant and it is also the first time of breastfeeding and the use of cow's milk in the first year. The results will subsidize strategy aimed at mothers of lower schooling, stimulating breastfeeding, supplementary feeding and iron supplementation and discouraging the use of cow's milk in the first year.

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INTRODUCTION

Anemia is a public health problem and children born prematurely are at a higher risk of developing it, corroborated by studies that show anemia in 26.5% to 34.5% of premature infants.¹⁻³ Micronutrient deficiencies stem from a broader context, and its occurrence is determined not only by biological factors, but also by socioeconomic and cultural conditions.^{4,5}

Premature infants are at risk because they have low reserves, they are exposed to an environment that exacerbates nutritional deficiencies, because of the lower success rates of their mothers on breastfeeding - they lack knowledge of their nutritional needs and the need to follow-up and care to be performed by a multiprofessional healthcare team.^{1,6,7}

Based on these premises, the present study aimed to analyze the factors that influence hemoglobin levels in the first year of life of preterm infants.

METHODOLOGY

This is a cross-sectional study with a cohort based on data obtained from the records of preterm infants followed at the State Center for Specialized Care (CEAE), in Viçosa-MG, registered from September 2010 to December 2015. CEAE's patient charts are semi-structured, a fact that made it possible to obtain reliably data for the present study.

Characteristics of the population and study site

São Sebastião Hospital (HSS), where all the births of Viçosa and vicinity occur, has been a reference for high-risk gestation since 2009; it has a human milk bank since 2005 and a neonatal intensive care unit since 2004.

The CEAE is the only referral service for the care of preterm infants in Viçosa and its vicinity, serving 20 small municipalities and a population of about 227,203 people. The annual number of live births in the municipality of Viçosa varied between 632 and 959, and its annual rates of prematurity varied between 8.8 and 9.9%. The health care for premature infants' follow-up is carried out by a multiprofessional team - composed of professionals in the fields of pediatrics, nursing, nutrition, psychology, physiotherapy and social care - and has an agreement with the Federal University of Viçosa (UFV). At the time of HSS hospital discharge, all preterm infants are referred to the CEAE for growth and development monitoring. The CEAE was inaugurated in September 2010, and totaled 190 premature infants until December 2015, whose annual admission ranged from 18 (period close to the opening) to 66 patients.

Inclusion and exclusion criteria

Inclusion criteria for the study were: to be born in the HSS with gestational age (GI) of less than 37 weeks, regardless

of birth weight, to maintain follow-up in the CEAE, to present a hemoglobin value record for the second semester of GA, and to complete outpatient follow-up up to a minimum of one year of CGA. Exclusion criteria were: medical records or hemoglobin data not found, severe malformations or hemolytic anemias.

Study variables

Premature: any newborn with a GA of less than 37 weeks. Chronological age (RCA): defined as the postnatal age. Corrected GA for prematurity (CGA): difference between gestational age at birth and mean duration of full term gestation (40 weeks).^{8,9}

Sociodemographic variables: maternal and paternal ages, maternal and paternal education (up to and from primary school) and family income in minimum wages (MW; < 2 MW and > 2 MW)¹⁰. Prenatal and perinatal period variables: type of delivery, gender, hospitalization in the neonatal intensive care unit (NICU), GA (in weeks and categorized as < 28 weeks, 28-31 weeks and ≥ 32 weeks for descriptive analysis; 32 weeks or ≥ 32 weeks for inferential statistics), birth weight (PN, in grams and categorized as < 1000g, 1000-1499g, 1500-2499g and ≥ 2500g for descriptive analysis; < 1500g and ≥ 1500g for inferential statistics). The adequacy of PN for GA was based on Fenton curves and was categorized as small for GA (SGA, values < 10th percentile) and not-SGA (values above the 10th percentile)^{8,11}.

Feeding at the first visit was categorized into breastfeeding (BF, considering exclusive or supplemented breastfeeding) and artificial feeding (AF).¹² The total BF duration was recorded, considering the CGA in months. The term "cow's milk" refers to cow's milk in natura and not to infant formula. Iron and polyvitamin supplementation followed the recommendations of the Brazilian Society of Pediatrics,¹³ with adjustments of the daily doses for body weight at the time of the consultations, on a monthly basis. The RCA was registered at the beginning of the use of iron and multivitamin supplementation. Zinc supplementation was not analyzed because it was implemented in 2014. We also documented hospital admissions.

The period evaluated was the first year of corrected gestational age (CGA) of preterm infants. For this period, the routine of the service foresees collection of blood samples for laboratory examinations in the first and second semesters or according to need. We analyzed hemoglobin levels (Hb) and the prevalence of anemia considering the second half of CGA, from the six months of RCA, because there were reference values from that age, and to avoid interferences from prematurity anemia. Thus, the anemia was characterized by an Hb value lower than 11.0 g/dL^{4,14}

Statistical analysis

The minimum sample size was defined using the variation coefficient obtained in the present study for the

hemoglobin levels variable (10.8%), considering a ten percent variation around the mean, obtaining the minimum sample size of 24 individuals for differences to be found at a 5% significance level.¹⁵

The study quantitative variables were presented as mean, standard deviation, median and interquartile range for descriptive analysis, according to the parametric or non-parametric distribution, which was checked by the Kolmogorov-Smirnov test. The qualitative variables were described in absolute and percentage values, considering the valid data.

A comparative analysis was performed between the premature babies that were included and those that were not included in the study by Pearson's chi-square test, Student's t-test or Mann-Whitney test. The median hemoglobin values of the preterm infants in the study were compared according to the explanatory variables by the Mann-Whitney test and Spearman's correlation test.

We applied multiple linear regression analysis to estimate the influence of explanatory variables on hemoglobin levels variation in the first year of preterm infants. We also carried out the logarithmic transformation of the dependent variable (hemoglobin), which did not present a parametric distribution. Univariate and later multivariate regression analyzes included explanatory variables that presented $p < 0.20$. The final model included the significant variables at the 0.05 level.

Multicollinearity was tested by the variance inflation factor (VIF), the Durbin-Watson test and residue analysis by predicted values. The Excel software (version 2010; Microsoft Office), Stata 9.1 and IBM-SPSS (version 23.0) were used for database manipulation, coding, typing and statistical analysis of data.

Ethical aspects

The Human Research Ethics Committee of the Federal University of Viçosa (CAAE 19676613.5.0000.5153) approved the study and it is part of the project "Growth, development and morbidity of infants and preterm infants, or with low birth weight". This study is in accordance with the Regulated Directives and Norms for Research Involving Human Beings, in accordance with Resolution No. 466, from December 12, 2012, of the National Health Council at the Ministry of Health, Brasília, DF. The confidentiality of the information of all the participants of the study was guaranteed.

RESULTS

Of the 190 records registered, seven were not found. Of these, 90 medical records were excluded because they did not record hemoglobin values for the second semester of CGA or because they did not complete outpatient follow-up until a minimum of one year of CGA. Data from 93

preterm infants were eligible for the study. When comparing preterm infants included and not included in the study, we noticed that there were no differences between their sociodemographic, perinatal and outpatient characteristics, except for the lower use of artificial feeding by the study population at the first outpatient visit (Table 1), thus avoiding selection bias in the present study.

In the study population, the median value of hemoglobin was 11.6 g/dL (10.8-12.3). Anemia occurred in 25.8% of premature infants ($n = 24$) and this group had a median hemoglobin of 10.2 g/dL (9.4-10.6). Among preterm infants who did not have anemia, the median hemoglobin was 11.9 g/dL (11.6-12.3).

The mean maternal and paternal ages were 26 and 29 years, respectively. As for schooling, 37.8% and 55.0% of the mothers and fathers did not complete elementary school, respectively. 54.3% of the families had an income of less than two minimum wages.

Of the preterm infants, 52.7% were males and 19.4% were born SGA. ($N = 7$), 18.3% ($n = 17$) and 74.2% ($n = 69$) of the preterm infants were born with less than 28 weeks, between 28-31 weeks and > 32 weeks. Their birth weight was less than 1000g, between 1000-1499g, between 1500-2499g and > 2500 g, respectively 6.5% ($n = 6$), 20.4% ($n = 19$), 52.7% ($n = 49$) and 20.4% ($n = 19$) of the preterm infants. After comparative tests between the strata, GA and PN were dichotomized into < 32 weeks and > 32 weeks, and < 1500 g and > 1500 g to proceed with the inferential analysis.

The first outpatient visit at the referral service occurred at 41 median weeks. The iron and multivitamin supplementation started at about one month of CRA. 82.8% of the preterm infants were breastfed at the first consultation, with a median duration of 5 months. It should be noted that 64.6% of the premature infants used cow's milk in their first year of life.

According to the data in Table 2, the lower hemoglobin levels were associated with lower maternal and paternal schooling, GA greater than 32 weeks, and cow's milk use in the first year.

In order to estimate the influence of the explanatory variables on the hemoglobin levels of preterm infants, those who presented $p < 0.20$ in the comparative tests were submitted to univariate linear regression analysis, and those that maintained a $p < 0.20$ were included in the multiple linear regression model (Table 3).

In the final model, low maternal schooling was associated with lower levels of hemoglobin, contributing with 8.7% of the variation in hemoglobin levels in the first year of CGA of premature infants (Table 4). VIF values = 1,000, Durbin-Watson test = 1,622 and residue analysis indicated that the model was well adjusted and that there was no multicollinearity or heteroscedasticity of the data.

Table 1. Comparative analysis among sociodemographic, perinatal and outpatient follow-up characteristics of the premature infants included and those not included in the study. Viçosa-MG, 2010-2015.

Variables	Study population (n = 93)		Excluded (n = 90)		p value
	n (%)	Mean + SD or Med (P25-P75)	n (%)	Mean + SD or Med (P25-P75)	
Male gender	49 (52,7)	-	53 (58,9)	-	0,457
Mother living in another city	40 (46,0)	-	21 (38,9)	-	0,458
GA < 32 weeks	24 (25,8)	-	27 (30,3)	-	0,514
BW < 1500g	25 (26,9)	-	29 (32,2)	-	0,517
Mother's age	88 (94,6)	26,4 + 6,8	86 (95,5)	26,4 + 6,9	0,99
Father's age	83 (89,2)	29,1 + 7,2	80 (88,9)	30,8 + 9,7	0,177
Born SGA	18 (19,4)	-	13 (14,4)	-	0,433
C-Section delivery	57 (61,3)	-	58 (64,4)	-	0,76
Admission to NNICU	61 (65,6)	-	59 (65,6)	-	1
Family income < 2 MW	44 (54,3)	-	35 (52,2)	-	0,869
Mother's schooling (< elementary)	34 (37,8)	-	35 (42,2)	-	0,641
Father's schooling (< elementary)	44 (55,0)	-	37 (50,0)	-	0,628
Mother's occupation (has a job)	39 (46,4)	-	42 (54,5)	-	0,345
CGA 1 ^a visit (weeks)	58 (62,4)	41,4 (39,5-43,0)	77 (85,6)	39,6 (37,3-46,4)	0,57
Total time of BF (CGA; months)	58 (62,4)	5,2 (2,6-6,1)	77 (85,6)	3,0 (2,0-5,2)	0,21
Age of iron use onset (CRA; months)	58 (62,4)	1,1 (0,3-1,6)	77 (85,6)	1,1 (0,4-1,3)	0,727
Age of polyvitamin use onset (CRA; months)	58 (62,4)	1,0 (0,2-1,2)	77 (85,6)	0,4 (0,2-1,1)	0,642
AF on first visit	16 (17,2)	-	32 (35,6)	-	0,005
CM in the first year	53 (64,6)	-	35 (81,4)	-	0,064
Hospitalization	9 (9,7)	-	10 (11,1)	-	0,593

SD, standard deviation; Med, median; P25, percentile 25; P75, percentile 75; GA, gestational age; BW, birth weight; SGA, small for gestational age; NNICU, neonatal intensive care unit; MW, minimum wage; CGA, corrected gestational age; CRA, chronological age; AF, artificial feeding; CM: cow's milk. Continuous variables: results plotted in mean and standard deviation (p value according to Student's t-test) or median and interquartile interval (p-value according to the Mann-Whitney test). Categorical variables: results in absolute values and frequencies (p-value according to the Pearson's Chi-square test).

Table 2. Median values of premature hemoglobin according to sociodemographic, perinatal and outpatient follow-up characteristics. Viçosa-MG, 2010-2015.

Variables	n (%)	Mean(± SD) or Med (P25 - P75)	Hemoglobin (g/dL) Med (P25 - P75)	p-value
<i>Maternal origin</i>				0,313 *
Another city	40 (46,0)	-	11,8 (11,0-12,3)	
Viçosa	47 (54,0)		11,3 (10,8-11,9)	
<i>Maternal schooling</i>				0,003 *
< elementary	34 (37,8)	-	11,2 (10,6-12,0)	
> elementary	56 (62,2)		11,6 (11,1-12,3)	
<i>Paternal schooling</i>				0,015 *
< elementary	44 (55,0)	-	11,2 (10,7-12,0)	
> elementary	36 (45,0)		11,7 (11,1-12,3)	
<i>Mother has job</i>				0,585 *
Yes	39 (46,4)	-	11,2 (10,8-12,3)	
No	45 (53,6)		11,8 (11,1-12,2)	
<i>Family income</i>				0,106 *
< 2 MW	44 (54,3)	-	11,4 (11,0-11,9)	
> 2 MW	37 (45,7)		11,6 (10,9-12,3)	

Continued **Table 2.**

<i>Maternal age</i>	93 (100)	26,4 + 6,8	-	0,473 **
<i>Paternal age</i>	93 (100)	29,1 + 7,2	-	0,882 **
<i>Gender</i>				0,560 *
Male	49 (52,7)	-	11,6 (10,9-12,3)	
Female	44 (47,3)		11,5 (10,9-12,1)	
<i>Type of delivery</i>				0,606 *
C-section	57 (61,3)	-	11,6 (10,8-12,2)	
Vaginal	36 (38,7)		11,6 (11,0-12,3)	
<i>Born SGA</i>				0,738 *
Yes	18 (19,4)	-	11,3 (10,8-12,0)	
No	75 (80,6)		11,6 (11,0-12,3)	
<i>NNICU admission</i>				0,065 *
Yes	61 (65,6)	-	11,6 (11,0-12,3)	
No	32 (34,4)		11,4 (10,7-11,9)	
<i>BW classification</i>				0,173 *
<1500g	25 (26,9)	-	12,0 (11,0-12,3)	
>1500g	68 (73,1)		11,5 (10,9-12,0)	
<i>GA classification</i>				0,013 *
< 32 weeks	24 (25,8)	-	12,3 (11,3-12,6)	
> 32 weeks	69 (74,2)		11,3 (10,8-11,9)	
<i>CGA 1st visit (weeks)</i>	92 (99,0)	41,4 (39,2-44,0)	-	0,496 **
<i>Feeding in the first visit</i>				0,661 *
AF	16 (17,2)	-	12,3 (12,0-12,9)	
BF	77 (82,8)		11,3 (10,8-11,9)	
<i>Total time in BF (CGA; months)</i>	75 (80,6)	5,2 (3,0-6,3)	-	0,541 **
<i>Age of iron use onset (CRA; months)</i>	85 (91,4)	1,1 (1,0-2,1)	-	0,707 **
<i>Age of polyvitamin use onset (CRA; months)</i>	82 (88,1)	1,1 (0,8-2,1)	-	0,473 **
<i>CM in the 1st year</i>				0,001 *
Yes	53 (64,6)	-	11,1 (10,7-11,9)	
No	29 (35,4)		11,9 (11,3-12,3)	
<i>Hospitalization in the 1st year</i>				0,543 *
Yes	9 (9,7)		11,0 (10,8-11,9)	
No	84 (90,3)		11,6 (10,9-12,3)	

The values refer to the total number of valid responses, and the absent data was not considered. SD, standard deviation; med, median; P25, percentile 25; P75, percentile 75. MW, minimum wage; SGA, small for gestational age; NNICU, neonatal intensive care unit; BW, birth weight; GA, gestational age, CGA, corrected gestational age; AF, artificial feeding; BF, breastfeeding; CRA, chronological age; CM, cow's milk. **p-value* by the Mann-Whitney test. ***p-value* by the Spearman's correlation test.

Table 3. Simple linear regression analysis of the hemoglobin values according to sociodemographic, perinatal and outpatient follow-up characteristics. Premature, Viçosa-MG, 2010-2015.

Variable	β	IC 95%	Valor <i>p</i>
Maternal schooling	-0,394	-0,149;-0,047	< 0,001
Paternal schooling	-0,266	-0,115;-0,008	0,024
Family income	-0,238	-0,111;-0,002	0,044
NNICU admission	0,178	-0,009;0,097	0,105
Birth weight	0,071	-0,040;0,078	0,52
Gestational age	0,142	-0,021;0,100	0,199
Cow's milk in the first year	-0,325	-0,139;-0,026	0,005

NNICU: neonatal intensive care unit. β : regression coefficient.

Table 4. Multiple linear regression final model regarding hemoglobin levels according to study variables. Premature infants, Viçosa-MG, 2010-2015.

Variable	β	IC 95%	Valor <i>p</i>
Maternal schooling	-0,295	(-0,134;-0,009)	0,026

R² = 0,087; β : regression coefficient.

DISCUSSION

The prevalence of anemia among premature infants in the first year of life in the study was significant (25.8%). Brazilian studies have shown anemia rates among preterm infants of very low birth weight ranging from 26.5% to 34.5%.^{2,3}

Anemia is a public health problem and premature infants are at higher risk for iron deficiency, and different factors are associated with its occurrence: its low reserves¹, exposure to the environment that exacerbates existing nutritional deficiencies after clamping the umbilical cord, the lower success rates of its mothers in breastfeeding¹⁶, the knowledge gaps concerning their nutritional needs⁶ and the need for follow-up and differentiated care by the healthcare team at the different levels of care.⁷ One of the factors that should be highlighted as an influence on the occurrence of anemia among premature infants is the low compliance to recommended supplementation. This effect is demonstrated in a cohort study performed with the premature infants of the service, among which the low compliance to supplementation is associated with a 2.5-fold higher chance for the occurrence of anemia.¹⁷

In the present study, low maternal schooling was associated with lower levels of hemoglobin, contributing with 8.7% of its variation in the first year of the CGA of preterm infants. The association between low maternal schooling and anemia has been demonstrated in the literature.¹⁸ Higher schooling is associated with higher income and availability of food at home, especially those rich in iron, as well as greater access to healthcare services. The fact that the higher level of education contributes to a better understanding of childcare; thus, a better nutritional offer is also added. Therefore, the lower level of schooling may be a risk factor for anemia.¹⁹⁻²¹

A previous study carried out with preterm infants in their first six months in our facilities demonstrates the association between low maternal schooling and low compliance to iron supplementation, impacting the occurrence of anemia.¹⁷ Micronutrient deficiencies have their genesis in a broad context, in which their occurrence is determined not only by biological factors, but also by the current socioeconomic and cultural conditions.^{4,5} In this aspect, it is important to highlight the need of healthcare professionals to work on health education strategies geared towards mothers with lower educational levels, intensifying practices of iron supplementation for premature infants.¹³ The training, cooperation and motivation of healthcare professionals are factors capable of positively influencing mothers with lower education.^{13,22}

In our study, although 82.8% of preterm infants were breastfed at their first visit, their median duration was 5 months. It should be noted that 64.6% of premature infants used cow's milk in their first year and that all premature infants who developed anemia in the first year used cow's milk. Attention should be paid to the realities of early weaning of premature infants, despite the acknowledged benefit of breast milk for children. There is already evidence that success rates in breastfeeding are lower in mothers of preterm infants, which is due to several factors, such as gastric immaturity, incoordination of suction-swallowing-breathing functions, and prolonged hospitalization.²³⁻²⁵ With breastfeeding interruption, milk formulas remain as alternatives to breast milk. However,

due to socioeconomic reasons, cow's milk is often initiated in the first year.²⁶ It is a fact that cows' milk fosters the development of anemia when compared to breastfeeding, because of its poor bioavailability and for causing gastrointestinal bleeding.²⁷⁻²⁹ The protective factor of exclusive breastfeeding in comparison to mixed breastfeeding is also demonstrated in the literature.¹⁹

As study limitations, this has a cross-sectional design, which is subject to information biases and enables only to evaluate prevalences and associations, not allowing to establish a causal relationship. In addition, only 8.7% of the variation in hemoglobin levels of preterm infants were explained by the study. We could not evaluate other factors that may impact on hemoglobin and anemia values, such as the age of introduction of supplementary feed, adequate consumption of meats and other food sources of iron and compliance to micronutrient supplementation.

As strengths, we highlight the adequate sample size and the absence of selection bias demonstrated by the fact that there were no significant differences between the characteristics of the studied population and the excluded premature infants, which enables inferences to be made to other populations of premature infants and, consequently, to guide other healthcare actions.

We conclude that, lower levels of hemoglobin among preterm infants were partially explained by lower maternal schooling. The prevalence of anemia among preterm infants was relevant, and we stress the short time of breastfeeding and the use of cow's milk in the first year.

Based on the results, we intend to foster the adoption of educational strategies aimed at mothers with less education in the healthcare network, seeking to strengthen the bond between the families of premature infants and healthcare units at all levels of care. The strategies to be adopted for the prevention of iron deficiency anemia include the encouragement of breastfeeding, the adequate introduction of complementary feeding, the fight against the use of cow's milk in the first year, and the intensification of iron supplementation for premature infants.

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