Original Articles

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Timing of clamping and factors associated with iron stores in full-term newborns

ABSTRACT

OBJECTIVE: To analyze the impact of timing of clamping and obstetric, biological and socioeconomic factors on the iron stores of full-term newborns.

METHODS: Cross-sectional study between October 2011 and July 2012 in which hematological parameters were evaluated for newborns in Viçosa, MG, Southeastern Brazil. It involved collecting 7 mL of umbilical cord blood from 144 full-term not underweight newborns. The parameters investigated were complete blood count, serum iron, ferritin and C-reactive protein. The time of umbilical cord clamping was measured using a digital timer without interfering in the procedures of childbirth. The birth data were collected from Live Birth Certificates and other information was obtained from the mother through a questionnaire applied in the first month postpartum. Analysis of multiple linear regression was then used to estimate the influence of biological, obstetrics and socioeconomic factors on the ferritin levels at birth.

RESULTS: The median ferritin was 130.3 µg/L (n = 129, minimum = 16.4; maximum = 420.5 µg/L), the mean serum iron was 137.9 µg/dL (n = 144, SD = 39.29) and mean hemoglobin was 14.7 g/dL (n = 144, SD = 1.47). The median time of cord clamping was 36 seconds, ranging between 7 and 100. The bivariate analysis detected an association between ferritin levels and color of the child, timing clamping of 60 seconds, type of delivery, the presence of gestational diabetes and *per capita* family income. In multivariate analysis, the variables *per capita* income, number of antenatal visits and length at birth accounted for 22.0% of variation in ferritin levels.

CONCLUSIONS: Iron stores at birth were influenced by biological, obstetric and social characteristics. Tackling anemia should involve creating policies aimed at reducing social inequalities, improving the quality of antenatal care, as well as implementing a criterion of delayed clamping of the umbilical cord within the guidelines of labor.

DESCRIPTORS: Anemia. Anemia, Iron Deficiency. Ferritins. Iron, deficiency. Umbilical cord. Infant, Newborn. Blood Cell Count. Constriction. Time Factors.

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INTRODUCTION

Iron deficiency in the first few months of a child's life is a public health problem. The consequences are both immediate and long term consequences, such as erythropoiesis and reduced capacity transport oxygen, effects on myelination and synaptogenesis, effects on growth and damage to enzymatic and metabolic functions and to the immune response system, as well as abnormalities in motor and cognitive development.[19,5] The World Health Organization (WHO) estimates that around 42.0% of pregnant women and 30.2% of women of childbearing age are anemic. This condition is related to the risk of premature birth, poorer Apgar scores, low birth weight, low concentrations of ferritin and development of childhood iron deficiency anemia.[1]

The factors which determine childhood iron deficiency are principally related to post natal speed of growth and to reserves of this mineral at birth.[19] These are acquired during the final trimester and are crucial for maintaining an adequate nutritional state of iron in the first months of life. Delayed clamping of the cord is recommended as a simple and low cost strategy for improving iron stores at birth and preventing childhood anemia.[7,20]

The literature differs concerning classifying what is early and what is delayed clamping. Early is considered to be immediately or within 15 seconds of birth, and delayed that performed after one, two or three minutes, or when the cord ceases to pulsate.[4,7,20] The WHO published a guide to integrated birth practices in 2007, which recommends that in completely reactive babies, clamping be conducted after umbilical cord pulsations cease, which corresponds to around three minutes.[6] The Brazilian Ministry of Health (MH) ratified this position in 2011 and the Brazilian Society of Pediatricians (SBP) recommended that full-term, healthy newborns be positioned on the mother's abdomen, at the same level as the placenta, for between one and three minutes, before cutting the umbilical cord.[a] There are no Brazilian studies evaluating the efficacy of cord clamping on hematological parameters at birth, the existence of which could assist in implementing SBP and MH recommendations. There are also few investigations into obstetric and socioeconomic factors which may be involved in determining iron stores at birth. This study is essential to instituting antenatal multi-sectorial actions and preventing childhood anemia.

Social determinants of iron deficiency anemia in childhood are frequently reported in the literature, showing that this lack is more than a biological phenomenon and has wider levels of determination. Iron deficiency anemia is a biological response to external stimuli which affect the individual's organism, be they macroeconomic, cultural or environmental phenomena, living and working conditions, social cohesion or lifestyle.[3,15]

The aim of this study was to analyze the impact of clamping time on iron stores in newborns.

METHODS

This was a cross-sectional study in which the hematological parameters of newborns in Viçosa, MG, Southeastern Brazil, were evaluated between October 2011 and July 2012.

The data were collected in the only maternity hospital in the municipality, Monday to Friday, during the period of research. Exclusion criteria were: residing in another municipality, multiple pregnancy, birth weight < 2,500 g, gestational age < 37 weeks and child having any kind of illness.

Sample size was calculated using data on live births available through the Live Birth Information System (Sinasc),[b] on residents in the municipality, with no congenital abnormalities, single, full-term pregnancies with birth weights ≥ 2,500 g (n = 555). As the percentage of low iron stores at birth was not known, due to a lack of cutoff points for this classification, an event frequency of 50.0% was used. An 8.0% level of acceptable variability was adopted in the estimated frequency and the 95% confidence level, giving 119 individuals. The final sample was calculated to be 143 babies, with 20.0% added to cover any losses.

Throughout the research period, a member of the research team remained on call at the hospital, waiting for women giving birth to be admitted. They were then invited to participate in the study. A nurse from the team collected the blood sample from the umbilical cord at birth.

The protocol of this study standardized clamping of at least 15 cm of cord, enough to collect 7 mL of blood, regardless of the type of delivery (natural or cesarean). Of this volume, 2 mL were placed in Vacuette® tubes with EDTA K3 for hemogram analysis and 5 mL were placed into Vacuette® tubes with separating gel to analyze ferritin.[20] The samples were immediately sent to the laboratory and analyzed.

The parameters evaluated in the umbilical cord blood were complete blood count, serum iron, ferritin and C-reactive protein (CRP). Blood counts were analyzed

[a] Almeida MFB, Guinsburg R. Programa de Reanimação Neonatal da Sociedade Brasileira de Pediatria: condutas 2011 [cited 2011 Jul 2]. Available from: http://www.sbp.com.br/pdfs/PRN-SBP-Reanima%C3%A7%C3%A3oNeonatalFinal-2011-25mar11.pdf
[b] Ministério da Saúde, DATASUS. Sistema de Informações sobre Nascidos Vivos – SINASC: Minas Gerais. 2010 [cited 2013 Apr 6]. Available from: http://tabnet.datasus.gov.br/cgi/deftohtm.exe?sinasc/cnv/nvmg.def

using the electrical impedance method, using the ABX Micros 60 instrument, serum iron using the Labtest Ferrozine® method and ferritin using the Immulite® automated chemiluminescent assay method. The photometric turbidimetric method was used to study PCR in serum. Children with an infection (PCR values > 1.0 mg/dL)[c] were excluded from the analysis of the ferritin results. The hematological parameters evaluated were not classified due to there being no international reference for the group studied. A digital timer was used to measure umbilical cord clamping time, without interfering in routine birth procedures. Counting was standardized as beginning when the baby's head was completely out of the vaginal canal of the cesarean opening, and stopped when the doctor performed the clamping with a surgical clamp. The cord clamping was performed by the obstetrician responsible for the birth, and the timing and the blood collection by a previously trained member of the research team.

Data on births were collected from the Live Birth Certificates (BC) in the hospital. Other information were obtained from the mother and child using a standardized semi-structured questionnaire.

Of the 144 babies assessed, nine had no ferritin analysis results due to problems in the laboratory (hemolysis or insufficient volume) and six were excluded, having elevated CRP, leaving 129 with complete results for ferritin levels at birth. Of these, 119 were interviewed and the questionnaire was completed.

The outcome studied was iron stores (ferritin) at birth, a known predisposing factor in childhood iron deficiency anemia, and was treated as a continuous variable/ descriptive analysis were performed and the Shapiro Wilk test was used to verify distribution of the variables. As the independent variable (ferritin) did not present normal distribution, the Kruskall-Wallis and Mann-Whitney tests were used to analyze the differences between median values and socioeconomic, biological and obstetric variables. The level of significance adopted to reject the null hypothesis was < 5% in all of the tests.

Multiple linear regression analysis was used to estimate the influence of the variables in the study on variation in ferritin stores at birth. The dependent variable (ferritin) did not present normal distribution, so quadratic transformation was performed. Univariate regression analysis was used to test each variable separately, aiming to select those to be integrated in the multiple model. Those with p < 0.20 were selected. Variables with p < 0.05 were significantly and independently associated and remained in the final model after adjustment.

Multicollinearity was tested using the variance inflation factor (VIF) and residue analysis using the estimated values. The presence of heteroscedasticity was investigated using the Breusch-Pagan-Godfrey test, adopting 5% as the level of significance for rejecting the null hypothesis (presence of heteroscedasticity).

Statistical analysis was performed using the Stata version 9.0 program.

This study was approved by the Ethics Committee for Research involving Human Beings of the *Universidade Federal de Viçosa* (213/2011). Participants signed a consent form.

RESULTS

The majority (51.4%) of the newborns were male, with most (71.3%) of the births being cesarean. The number of antenatal appointments varied between one and 15 (mean = 7.6; sd = 2.2) and 14.1% of the women did not have the minimum six antenatal appointments established by the Ministry of Health.[d] Iron supplements were taken during pregnancy by 96.4% of the women, mean birth weight was 3,326 g (sd = 387.4 g) and the mean Apgar score at five minutes was 9.2 (sd = 0.7).

Median maternal age was 26 (minimum 13, maximum 40), 12.6% of the mothers were adolescents (< 20 years old). The number of children per family varied between one and six and it was the first birth for 50.0% of the women. Mean maternal schooling was 9.5 years of studies (sd = 3.6) and median *per capita* income was R$ 325.00, equivalent to 0.48 minimum wages. Most of the women were in socioeconomic class C (58.7%), were non-white (black or mixed race – 53.4%), living in the urban zone (95.0%) and users of public health care services (65.2%).

Table 1 shows the distribution of the hematological parameters evaluated to verify nutritional state for iron.

Median umbilical cord clamping time was 36 seconds; with the minimum time 7 and the maximum 100 seconds; 77.0% of children's umbilical cords were clamped for < 60 seconds. The clamping time of babies born through cesarean was significantly lower than those with normal births (38.6 and 48.6 seconds, respectively, p = 0.04). Although ferritin levels at birth are related to clamping time (Table 2), they did not differ according to type of birth.

Clamping time was positively correlated with Mean Corpuscular Volume (MCV, r = 0.19, p = 0.02) and Mean Corpuscular Hemoglobin (MCH, r = 0.22,

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[c] Report of a Joint World Health Organization/ Centers for Disease Control and Prevention Technical Consultation on the Assessment of Iron Status at the Population Level; 2004 April 6-8; Geneva, Switzerland. Assessing the iron status of populations. 2.ed. Geneva: WHO; 2007.
[d] Ministério da Saúde, Secretaria de Atenção à Saúde, Departamento de Atenção Básica. Atenção ao pré-natal de baixo risco. Brasília (DF): Editora do Ministério da Saúde; 2012. (Série A. Normas e Manuais Técnicos. Cadernos de Atenção Básica, 32).

Table 1. Central trends and variability in newborns' hematological parameters. Viçosa, MG, Southeastern Brazil, 2012. (N = 144)

Parameters	P5	P10	P25	Median	P75	P90	P95	Mean	SD
Ferritin (µg/L)[a]	34.1	42.4	74.9	128.7	189.6	241.0	303.0	142.5	86.3
Serum iron (µg/dL)	75.0	90.0	110.0	139.0	160.0	190.0	207.0	137.9	39.3
Hemoglobin (g/dL)	12.7	13.2	13.8	14.7	15.7	16.7	17.4	14.7	1.5
Erythrocytes (millions/mm³)	3.6	3.8	4.0	4.3	4.7	4.9	5.0	4.3	0.5
Hematocrit (%)	39.0	40.0	42.0	44.8	48.0	51.2	52.9	44.9	4.6
MCV (fL)	96.4	98.1	100.8	104.0	107.0	110.5	114.3	104.0	5.2
MCH (pg)	31.4	31.8	32.8	34.1	35.3	36.7	37.7	34.2	1.9
CMCH (g/L)	32.0	32.3	32.6	32.9	33.2	33.3	33.5	32.8	0.4
RDW (%)	14.3	14.5	15.0	15.5	16.0	16.8	17.2	15.5	1.0

SD: standard deviation; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; CMCH: Mean Corpuscular Hemoglobin Concentration; RDW: Red blood cell distribution width
[a] N = 129

$p = 0.009$). Babies with clamping time < 60 seconds had lower MCV and MCH values (both $p = 0.002$), with no difference for hemoglobin, hematocrit, erythrocytes, serum iron and other blood indices.

Mean levels of ferritin were lower in babies with umbilical cord clamping < 60, those who were non-white, those whose mothers had gestational diabetes and those with cesarean births (Table 2).

There was no association ($p > 0.05$) between ferritin stores and the following factors: maternal schooling, socioeconomic class, type of water supply in the home, zone of residence, use of public health care services, having antenatal appointments with the public health care service, receiving the Bolsa Família benefit, interval between pregnancies, smoking or drinking during pregnancy, previous miscarriages, previous premature or low birth weight children, taking supplement during pregnancy, gestational age, anemia during pregnancy, maternal age, marital status or first pregnancy.

Table 3 shows the results of the simple linear regression analysis, in which the included variables presented $p < 0.20$ and were separated hierarchically into blocks.

In the final multiple linear analysis, determining factors in low iron stores at birth were, being in the lowest *per capita* income tertile, greater length at birth and fewer antenatal appointments (Table 4). This model contributed to 22.0% of variation in ferritin levels.

VIF value were 1.35 and $p = 0.86$, using the Breusch-Pagan-Godfrey test, both indicating that there was no multicollinearity or heteroscedasticity in the data and that the model had good fit.

DISCUSSION

There was evidence of influence of biological, obstetric and social factors on iron stores at birth. Social determinants of health can affect the individual's nutritional state of iron at all stages of their life: in uterus, in childhood and in adulthood. If not dealt with, this can create an intergenerational cycle, imprisoning the individual in a situation of food insecurity and nutritional deficiency, producing babies with the same deficiency, who grow into anemic children. Brazil is a country of great social inequality (Gini index of 0.526) and a large number of individuals living in poverty. Around 8.5% of Brazilians (16.3 million) live in extreme poverty, i.e., with mean *per capita* income below R$ 70.00[e] (US$169.05).[f] When inequalities are stratified according to race, non-whites are shown to have worse living and socioeconomic conditions, the result of unequal access to better paid occupations.[g]

There is little literature concerning the influence of social determinants on hematological parameters at birth, which makes comparison of this study difficult. However, low *per capita* income is related to higher risk of anemia in breastfeeding babies.[12,18]

Babies with umbilical cord clamping of > 60 seconds had higher mean ferritin levels at birth, similar to what has been shown in other studies evaluating different parameters of the nutritional state for iron at birth. In the study by Shirvani et al, 100 Iranian children were divided into two groups, using a cutoff point of 15 seconds. Forty-eight hours after birth, analysis showed higher levels of hemoglobin in the group with clamping > 15 seconds.[17] In a study conducted in Argentina, evaluating full-term newborns six hours

[e] Instituto Brasileiro de Geografia e Estatística. Censo Demográfico 2010: resultados sobre rendimentos – universo [cited 14 Sept 2012]. Available from: http://www.ibge.gov.br/home/estatistica/populacao/censo2010/rendimentos_preliminares/default_rendimentos_preliminares.shtm
[f] Dollar = 2.415 in 2014.
[g] Instituto de Pesquisa Econômica Aplicada. PNAD 2007: primeiras análises. Brasília (DF); 2008. (Comunicado da Presidência, 9. Série Pobreza e Mudança Social, 1).

Table 2. Means and medians of ferritin levels (μg/L) in newborns, according to socioeconomic, biological and obstetric variables. Viçosa, MG, Southeastern Brazil, 2012.

Variable	n	%	Mean	SD	Median	Minimum	Maximum	p
Clamping time								
< 60 seconds	97	77.0	134.3	88.3	110.0	16.4	406.0	0.01[a]
≥ 60 seconds	29	23.0	169.2	80.6	165.2	62.6	400.1	
Gestational diabetes								
Yes	6	5.6	85.4	45.4	82.8	26.0	420.5	0.04[a]
No	101	94.4	151.8	86.3	140.0	16.4	406.0	
Skin color (baby)								
White	59	56.2	161.4	84.6	150.0	28.4	374.0	0.04[a]
Non-white	46	43.8	131.1	86.7	109.8	16.4	406.0	
Skin color (mother)								
White	43	43.4	156.9	74.1	151.0	26.0	339.0	0.12[a]
Non-white	56	56.6	138.1	86.0	121.0	16.4	406.0	
Per capita income								
1st tertile (62.2 to 207.3)	30	29.1	129.7	72.6	120.0	37.9	406.0	0.05[b]
2nd tertile (212.5 to 405.0)	35	34.0	136.7	93.5	121.0	16.4	374.0	
3rd tertile (414.6 to 2,250.0)	38	36.9	172.4	88.2	150.5	38.8	400.1	
Number of antenatal appointments								
< 6	15	15.5	164.1	78.9	174.0	37.9	374.0	0.17[a]
≥ 6	82	84.5	142.4	82.1	129.5	16.4	406.0	
Type of delivery								
Normal	37	29.1	165.8	93.0	159.3	37.9	420.5	0.02[a]
Cesarean	90	70.8	133.0	82.9	118.2	16.4	400.1	
First birth								
Yes	46	46.0	130.5	91.6	109.3	24.8	400.1	0.06[a]
No	54	54.0	155.5	81.3	150.7	16.4	406.0	
Anemia in pregnancy								
Yes	31	30.7	128.1	85.9	109.0	24.8	400.1	0.07[a]
No	70	69.3	151.6	79.0	150.7	16.4	406.0	

SD: standard deviation
Values in bold are statistically significant
[a] Mann-Whitney Test.
[b] Kruskal-Wallis Test.

after birth, times of 15 seconds, one and three minutes were tested. The authors observed lower prevalence of low hematocrit levels in the groups with times of one and three minutes, compared with the 15 second group.[4] Emhamed et al compared hemoglobin levels in 104 Lebanese children 24 hours after birth, using clamping time of 10 seconds (early) and after cessation of umbilical cord pulsations (delayed) as criteria. The authors found better hemoglobin levels in babies with delayed clamping.[9]

Considering the findings of this study and those in the literature, it is deemed that approximately 80.0% of placental transfusion occurs in the first minute after birth, and a time of at least one minute needs to be adopted as a fundamental strategy in combatting iron deficiency anemia in countries with high prevalence of this deficiency. It is a practical and low cost measure, of proven efficacy. Delayed clamping in full-term babies may encourage increased blood volume of between 25 and 35 mL/kg of body mass, equivalent to 46 to 60 mg of iron from hemoglobin. This would be enough to maintain the necessary levels of iron for the first three months of life, which could make a great difference to the first six months of life of vulnerable babies.[7]

The clamping time of one minute, proposed by the SBP is capable if encouraging positive results in full-term newborns' iron stores, proving the need for this criterion to be effectively implemented in Brazilian

Table 3. Simple linear regression analysis of ferritin levels in newborns according to socioeconomic, biological and obstetric variables. Viçosa, MG, Southeastern Brazil, 2012.

Variable	β	95%CI	p
Umbilical cord clamping time	0.03	0.001;0.06	0.03
Number of antenatal appointments	0.36	0.07;0.65	0.01
Length at birth	-0.42	-0.77;-0.08	0.01
Type of delivery (normal/cesarean)	-1.42	-2.78;-0.05	0.04
Gestational diabetes	-2.75	-5.55;0.05	0.05
Non-white skin color (baby)	-1.24	-2.60;0.11	0.07
Per capita income (tertiles)	-0.76	-1.62;0.09	0.08
First birth	-1.14	-2.49;0.20	0.09
Anemia in pregnancy	-1.11	-2.56;0.33	0.13
Non-white skin color (mother)	-0.93	-2.29;0.41	0.17

β: coefficient of regression

maternity hospitals. Other studies are needed to evaluate the impact of times of one and three minutes, recommended by the SBP[b] and by the WHO,[6] respectively, on newborn health.

Women with gestational diabetes gave birth to babies with lower ferritin levels at birth, according to the bivariate analysis. This complication is a risk factor for iron deficiency in newborns, since it leads to elevated uterine hypoxia with compensatory erythropoiesis and increased utilization of iron stores.[11] However, the presence of gestational diabetes may be associated with the higher incidence of cesarean births in this study, which may bias the results.

The number of antenatal appointments is independently associated with ferritin levels at birth, as in other studies.[14,16] The Brazilian Ministry of Health recommends at least six antenatal appointments and anemia should be investigated at two stages, at least: as early as possible and in the 28th week. Women are recommended to take a prophylactic iron supplement from the 20th week until the third antenatal month.[h] As 96.4% of women reported having taken the supplement, and 86.0% had at least the minimum number of appointments, the flaw may be in the quality of the antenatal appointments, whether due to lack of hemograms in the final trimester or to the absence of guidance on taking iron sulfate. Rather than increasing the number of antenatal appointments, it is essential that the quality of these appointments is improved. Women with poorer living conditions, lower schooling and risk behavior merit special attention, as they are most in need and are those who are most frequently found at the margins of health care.[10]

Length at birth was independently correlated with ferritin levels at birth. This reduction in ferritin may be caused by the child's increased length, as, the greater the length of the body, the greater the volume of blood and the greater the demand for iron to produce hemoglobin, as well as the greater need for iron for metabolic functions and transport. Another hypothesis is that smaller babies may have suffered possible intra-uterine nutrition restriction, which did not affect iron levels due to the maternal adaptive mechanism, which maintains adequate iron transport for the baby.

Studies have found a link between lower height and anemia in older children.[8,13] As height is strongly influenced by social factors, a child born smaller, which then finds itself in an unfavorable social environment may, as well as remaining shorter, suffer from deterioration in its nutritional state for iron.

Percentage distribution of hematological parameters at birth can be supported by further studies to determine reference values at birth. A greater sample size would be required for this.

Iron deficiency anemia is preventable. By identifying the scale of this problem and associating risk factors, it is possible to intervene directly. Intervention programs over the last few decades, such as fortifying foods and the prophylactic supplement program, have resulted in a worldwide decrease in prevalence.[2]

Although activities such as providing supplements and fortifying food are extremely relevant to improving the population's nutritional state for iron, combating anemia should not be limited to these measures. Seeking causality for this deficiency implies examining the different levels of determination of the phenomenon, levels concerned with biological and social relationships which define the health-disease process. It is necessary to prioritize combating health inequalities,

Table 4. Multiple linear regression analysis of ferritin levels in newborns according to socioeconomic, biological and obstetric variables. Viçosa, MG, Southeastern Brazil, 2012.

Variável	β	95%CI	β_{aj}	95%CI
Per capita income (1ª tertile)	-0.76	-1.62;0.09	-2.43	-4.42;-0.45
Length at birth	-0.42	-0.77;-0.08	-0.48	-0.66;-0.01
Number of antenatal appointments	0.36	0.07;0.65	-0.36	-0.69;-0.04

R^2 = 0.22; β: coefficient of regression

improve income distribution, increase access to, and improve the quality of antenatal care, as well as implementing the criterion of delayed umbilical cord clamping in national directives concerning giving birth.

The main limitation of this study is the lack of prospective investigation of gestational anemia in the mothers. We suggest measuring hemoglobin and ferritin levels in the mother, at different antenatal periods, as well as better clarifying mother-fetus transport of iron in future studies.

This study was unprecedented in Brazil regarding the influence of clamping time on iron stores at birth. There is little literature investigating factors related to hematological parameters at birth, and what there is focuses on perinatal, obstetric and maternal factors, rather than looking at social determinants involved with low iron stores at birth. It is recommended that this type of study be applied in developing countries where social determinants are highly relevant to anemia.

ACKNOWLEDGEMENTS

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