

Hemoglobinopathies in newborns from Salvador, Bahia, Northeast Brazil

Hemoglobinopatias em recém-nascidos de Salvador, Bahia, Nordeste do Brasil

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Abstract

Hemoglobinopathies are hereditary disorders of the hemoglobin molecule with a high prevalence worldwide. Brazil has a prevalence of 0.1 to 0.3% of newborns with sickle cell anemia and 20.0 to 25.0% of heterozygous α_2 thalassemia among African Brazilians. In the present study, we investigated the presence of variant hemoglobins and $\alpha_2^{3.7\text{ Kb}}$ and $\alpha_2^{4.2\text{ Kb}}$ thalassemia in newborns from Salvador, Bahia, Brazil. Samples of umbilical cord blood from a total of 590 newborns were analyzed, of which 57 (9.8%) were FAS; 36 (6.5%) FAC; one (0.2%) SF; and five (0.9%) FSC. One hundred fourteen (22.2%) newborns had $\alpha_2^{3.7\text{ Kb}}$ thalassemia, of whom 101 (19.7%) were heterozygous and 13 (2.5%) homozygous, showing statistical significance for hematological data between newborns with normal α genes and $\alpha_2^{3.7\text{ Kb}}$ thalassemia carriers. The $\alpha_2^{4.2\text{ Kb}}$ thalassemia was not found. Frequencies found in the present study confirm that hemoglobinopathies are a public health problem in Brazil, emphasizing the need for neonatal screening and genetic counseling programs.

Hemoglobinopathies; Sickle Cell Anemia; Thalassemia; Newborn Infant

Introduction

Hemoglobinopathies are genetic globin gene disorders, characterized by the presence of variant hemoglobin and a decrease or absence of globin chain synthesis, known as thalassemia ^{1,2}.

Hemoglobin S is the most common variant hemoglobin, and results from a single amino acid substitution of valine for glutamic acid at the sixth position of the β -globin chain; sickle cell anemia carriers are characterized by homozygosity of S hemoglobin. The hemoglobin S gene has a high frequency among Africans and African descendents, as well as in India, Greece, and the United States ³. Primary studies in Brazil revealed a high prevalence of hemoglobin disorders. The sickle cell trait (AS) was reported in 6.6% of blacks in the State of São Paulo, in Southeast Brazil ⁴. When the study was extended to the general population, frequencies of 2.7% of AS and 0.09% of sickle cell disease (0.07 % HbSS and 0.02% HbSC) were observed. These frequencies varied widely according to the degree of racial admixture in the different regions of the country. In the South of Brazil, a frequency of 1.2% of HbS gene was shown among newborns ⁵. On the other hand, in the Northeast, frequencies of 5.1% of sickle cell trait (FAS) and 0.2% with sickle cell disease (FSC) were reported among newborns in the State of Pernambuco ⁶ and the State of Bahia, the frequency of AS genotype varies from 7.4%

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to 15.7%, according to the population group studied ⁷.

Hemoglobin C (HbC) is a variant hemoglobin in which lysine replaces glutamic acid at the sixth amino acid position of the β -globin chain ². HbC has a prevalence of 3% among African-Americans and about 1-3% among Puerto Ricans ^{8,9}. In Brazil, HbC is the second most common variant hemoglobin and has been found around 2.2% to 5.2% when the heterozygous genotype (AC) has been considered in Bahia ⁷.

Thalassemia syndromes are found worldwide, especially α - and β -thalassemia ^{10,11}. In Southeast Brazil, a frequency of 1.3% of β -thalassemia trait and 0.1% of β -thalassemia major was reported for the general population ¹², while α_2 -thalassemia by a 3.7 kb DNA deletion ($\alpha_2^{3.7\text{Kb}}$ -thalassemia) varied from 20.0% to 25.0% in black populations ¹³, and Borges et al. ¹⁴ found 49.9% of α -thalassemia in adult outpatients seen at the University of Campinas Hospital with microcytosis and hypochromia without anemia. In the Northeast, $\alpha_2^{3.7\text{Kb}}$ -thalassemia was investigated in 106 pregnant women with AC and AA hemoglobin pattern, showing a 21.7% heterozygous and 0.9% homozygous rate for this alteration ¹⁵.

The Consensus Conference Panel convened by the National Institutes of Health (United States) in 1987 recommended newborn screening for hemoglobinopathies in order to decrease morbidity and mortality associated with sickle cell disease. *Streptococcus pneumoniae* is a common cause of death in sickle cell patients and the early diagnosis of sickle cell disease could alert clinicians to potential clinical complications of the disease and allow prompt clinical care and prophylactic therapy with vaccines and antibiotics ^{16,17}.

In Brazil, not all States have consolidated neonatal screening programs for hemoglobinopathies, despite a ruling mandating them by the Ministry of Health in June 2001 (Portaria GM/MS 822 – Diário Oficial da União 2001; 7 jun). The sickle cell disease screening program in Minas Gerais State was considered a model for South America by Serjeant ¹⁸. This program found 3.2% of screened babies with sickle cell trait, 1.3% heterozygous for HbC, and 0.08% with sickle cell disease. Other States have developed excellent newborn screening programs, such as Bahia, Pernambuco, Maranhão, the Federal District (Brasília), São Paulo, Rio de Janeiro, Espírito Santo, Santa Catarina, Paraná, Mato Grosso do Sul, and Rio Grande do Sul

(Technical Advisory Group on Neonatal Screening, Ministry of Health, Brazil). In California (United States), a newborn hemoglobinopathy screening program evaluated two million newborns and found 492 with sickle cell disease, while 290 had hemoglobinopathy SS, 143 SC, and 47 S β + thalassemia ¹⁹. The screening programs' implementation is an important step for increasing survival, reducing hospitalization, and minimizing expenses associated with sickle cell disease ^{20,21}. Based on the high frequency of hemoglobinopathies in Salvador, Bahia, Brazil, we investigated the presence of variant hemoglobin and $\alpha_2^{3.7\text{Kb}}$ and $\alpha_2^{4.2\text{Kb}}$ thalassemias among newborns babies in order to identify potential associations between hemoglobin abnormalities and hematological characteristics.

Material and methods

Casuistic

From February to June 2000, a cross-sectional epidemiological study analyzed 590 neonates delivered by vaginal birth at the Tsylla Balbino Maternity Clinic located in Salvador, Bahia, Brazil. Information about newborns and gestational age was obtained from mothers by a questionnaire and patient records.

Newborns with gestational age less than thirty-eight weeks were considered premature ²². Racial composition was determined by observation of facial characteristics and color of mamillae and scrotum ²³.

Samples

Umbilical cord blood samples were collected by midwives under supervision of maternity clinic physician staff. After clamping, blood was drawn into a Vacutainer tube (Becton-Dickinson – Corkeyville, Maryland, United States) containing EDTA and transported to the Pathology and Molecular Biology Laboratory, Gonçalo Moniz Research Center, Oswaldo Cruz Foundation (FIOCRUZ). Hematological analysis was performed by automated cell counter (Coulter T-890 – Coulter Corporation, Florida, United States) and DNA was isolated from leukocytes (GFX™ Genomic Blood DNA Purification KIT – Amersham Pharmacia Biotech, United States).

Hemoglobin profile

Hemoglobin profile was analyzed by High Performance Liquid Chromatography – HPLC Variant Hemoglobin Testing System (Bio-Rad Laboratories – California, United States). HPLC analyses were interpreted by comparing peak retention times with those obtained for the AFCS control, utilizing the sickle cell kit for hemoglobin screening in newborns.

α_2 -Thalassemia

The $-\alpha_2^{3.7\text{Kb}}$ and $-\alpha_2^{4.2}$ deletions were identified by polymerase chain reaction (PCR), using specific primers and reaction conditions as previously described²⁴.

Statistical analysis

The statistical analyses were conducted in the Epi Info software, version 6.04. Statistical significance was established at $P \leq 0.05$.

Ethical considerations

The study was approved by the Institutional Review Board/Ethics Committee of the Gonçalo Moniz Research Center, FIOCRUZ, after the newborn's parent or guardian had signed the informed consent form.

Results

The Tsylla Balbino Maternity Clinic is the largest public maternity ward in Salvador. During the study period, 2,958 children were born and 590 newborns (19.9% of total birth cohort) were analyzed, after statistical sample calculation. Of those analyzed, 63 (10.8%) had premature

delivery and 523 (89.2%) had normal gestational age; 296 (51.3%) were female and 281 (48.7%) male. Racial composition was determined for 578 babies: 98 (17.0%) were classified as white, 309 (53.4%) as mulatto, and 171 (29.6%) as black. Mean weight was 3.152 kg (± 0.530).

Hematological data and hemoglobin profiles

Hemoglobin profiles were determined in 581 of the 590 newborns: 480 (82.6%) had the normal profile FA, and 101 (17.4%) presented variant hemoglobins, of which 57 (9.8%) were heterozygous for HbS (FAS), and 38 (6.5%) were heterozygous for HbC (FAC). One (0.2%) baby was homozygous for HbS (FS) and five (0.9%) were double heterozygous for HbS and HbC (FSC).

There was no statistical difference in the gestational ages of newborns with respect to normal and variant hemoglobin patterns. The racial distribution and hemoglobin types of 502 newborns are shown in Table 1. Of the 47 newborns found to have the FAS pattern, 24 (51.1%) were mulatto, 15 (31.9%) were black, and eight (17.0%) were white.

Newborns with normal and variant hemoglobin had statistically similar hematological characteristics, except for the FA and FAC groups, which were statistically different for PVC, RBC, and MCHC values, with p-values of 0.031, 0.003, and 0.0009, respectively (Table 2).

α_2 thalassemia analyses

Of the 590 newborns, 514 were analyzed for α_2 thalassemia: 400 (77.8%) had normal α -genes ($\alpha\alpha/\alpha\alpha$) and 101 (19.7%) were heterozygous (α/α) and 13 (2.5%) homozygous ($-\alpha/\alpha-$) for the $\alpha_2^{3.7\text{Kb}}$ deletion. There was no statistical difference between presence of $\alpha_2^{3.7\text{Kb}}$ thalassemia and premature delivery.

Table 1

Ethnic characteristics and hemoglobin frequencies in newborns from the Tsylla Balbino Maternity Clinic. Salvador, Bahia, Brazil.

Racial group	Hemoglobin profile									
	FA		FAS		FAC		FSC		FS	
	n	%	n	%	n	%	n	%	n	%
White (n = 86)	76	18.2	8	17.0	2	6.0	–	–	–	–
Mulatto (n = 274)	227	54.4	24	51.1	19	57.6	3	75.0	–	–
Black (n = 142)	114	27.4	15	31.9	12	36.4	1	25.0	1	100.0
Total (n = 502)	417	100.0	47	100.0	33	100.0	4	100.0	1	100.0

Among the 114 newborns identified as $\alpha_2^{3.7Kb}$ thalassemia carriers, 21 (18.4%) were whites, 57 (50.0%) were mulattos, and 36 (31.6%) blacks. Table 3 shows the statistical analysis of hematological parameters of newborns with normal α -genes and $\alpha_2^{3.7Kb}$ thalassemia carriers.

Analysis of hemoglobin profiles and presence of $\alpha_2^{3.7Kb}$ thalassemia was performed on 451 of 590 newborns. Of the 377 newborns with FA pattern, 295 (78.2%) had normal α -genes, 72 (19.1%) were heterozygous, and ten (2.7%) homozygous.

Of the 43 newborns with FAS profile, 32 (74.4%) had normal α -genes, nine (20.9%) were

heterozygous, and two (4.7%) were homozygous; of the 27 newborns with pattern FAC, 22 (81.5%) had normal α -genes and five (18.5%) were heterozygous; among the four newborns with FSC pattern, three (75.0%) had normal α -genes and one (25.0%) was heterozygous for $\alpha_2^{3.7Kb}$ thalassemia. The newborn with FS pattern had normal α -genes.

Table 4 shows the hematological data from newborns with normal and variant hemoglobin genotypes, as well as for $\alpha_2^{3.7Kb}$ thalassemia carriers.

There was no statistical difference in the hematological characteristics of newborns with

Table 2

Hematological data and hemoglobin profile among newborns from the Tsylla Balbino Maternity Clinic. Salvador, Bahia, Brazil.

Hemoglobin pattern	PCV (%)	RBC ($\times 10^6$ /mL)	Hematological data (mean \pm SD)			
			Hb (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)
FA (n = 480)	46.55 (± 6.11)	4.31 (± 0.56)	14.76 (± 1.79)	108.42 (± 6.20)	34.53 (± 5.70)	31.82 (± 2.68)
FAS (n = 57)	45.38 (± 5.97)	4.19 (± 0.53)	14.57 (± 1.92)	108.40 (± 6.42)	34.89 (± 2.80)	32.17 (± 1.57)
	p = 0.18*	p = 0.15*	p = 0.44 *	p = 0.99*	p = 0.38*	P = 0.66**
FA (n = 480)	46.55 (± 6.11)	4.31 (± 0.56)	14.76 (± 1.79)	108.42 (± 6.20)	34.53 (± 5.70)	31.82 (± 2.68)
FAC (n = 38)	43.37 (± 7.73)	4.02 (± 0.71)	14.44 (± 1.72)	107.95 (± 4.11)	36.78 (± 6.84)	34.13 (± 6.68)
	p = 0.031**	p = 0.003*	p = 0.27*	p = 0.25**	p = 0.23**	p = 0.0009**
FA (n = 480)	46.55 (± 6.11)	4.31 (± 0.56)	14.76 (± 1.79)	108.42 (± 6.20)	34.53 (± 5.70)	31.82 (± 2.68)
FSC (n = 5)	48.75 (± 5.56)	4.45 (± 0.47)	14.73 (± 1.58)	109.55 (± 5.45)	33.45 (± 4.98)	30.55 (± 4.20)
	p = 0.47*	p = 0.62*	p = 0.96*	p = 0.72*	p = 0.56*	p = 0.34*

* ANOVA; ** Kruskal-Wallis H.

PCV = Packed cell volume; RBC = Red blood cells; Hb = Hemoglobin; MCV = Mean cell volume; MCH = Mean cell hemoglobin; MCHC = Mean cell hemoglobin concentration.

Table 3

Hematological data and α genes status among newborns from the Tsylla Balbino Maternity Clinic. Salvador, Bahia, Brazil.

Hematological data (mean \pm SD)	Normal α -genes (n = 400)	$-\alpha_2^{3.7Kb}$ thalassemia carriers (n = 114)	P-value
PCV (%)	46.98 (± 5.97)	44.90 (± 6.72)	0.002*
RBC ($\times 10^6$ /mL)	4.26 (± 0.54)	4.41 (± 0.65)	0.002**
Hb (g/dL)	14.09 (± 1.69)	14.26 (± 1.72)	0.00009*
MCV (fL)	110.23 (± 4.43)	102.78 (± 6.25)	< 0.00001**
MCH (pg)	35.36 (± 3.78)	32.64 (± 3.49)	< 0.00001**
MCHC (g/dL)	32.10 (± 3.14)	31.76 (± 2.87)	0.30*

* ANOVA; ** Kruskal-Wallis H.

PCV = Packed cell volume; RBC = Red blood cells; Hb = Hemoglobin; MCV = Mean cell volume; MCH = Mean cell hemoglobin; MCHC = Mean cell hemoglobin concentration.

Table 4

Hematological data and hemoglobin profile among newborns with $\alpha_2^{3.7Kb}$ thalassemia from the Tsylla Balbino Maternity Clinic. Salvador, Bahia, Brazil.

Hemoglobin pattern	PVC	RBC ($\times 10^6/\text{mL}$)	Hematological data (mean \pm SD)			
			Hb (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)
FA (n = 82)	45.47 (\pm 5.96)	4.51 (\pm 0.55)	14.38 (\pm 1.47)	102.09 (\pm 6.39)	32.08 (\pm 2.67)	31.41 (\pm 1.67)
FAS (n = 11)	41.99 (\pm 8.15)	4.04 (\pm 0.82)	13.63 (\pm 2.41)	104.41 (\pm 4.18)	34.16 (\pm 4.03)	32.66 (\pm 3.16)
	p = 0.09*	p = 0.01*	p = 0.47**	p = 0.25*	p = 0.03*	p = 0.29**
FA (n = 82)	45.47 (\pm 5.96)	4.51 (\pm 0.55)	14.38 (\pm 1.47)	102.09 (\pm 6.37)	32.08 (\pm 2.67)	31.41 (\pm 1.67)
FAC (n = 5)	39.10 (\pm 11.79)	3.75 (\pm 10.9)	13.56 (\pm 2.30)	103.92 (\pm 4.01)	39.09 (\pm 9.30)	36.66 (\pm 10.15)
	p = 0.11**	p = 0.11**	p = 0.25*	p = 0.53*	p = 0.031**	p = 0.018**

* ANOVA; ** Kruskal-Wallis H.

FA and FSC hemoglobin profile and thalassemia $\alpha_2^{3.7Kb}$ carriers.

Discussion

The frequencies of variant hemoglobin found in this study (Hb FAS 9.8%; Hb FAC 6.5%; FS 0.2%, and FSC 0.9%) are the highest described in Brazil. The high frequencies of variant hemoglobin are probably due to the high rate of racial admixture in the Bahian population, with a strong African gene component introduced by the African slave trade in Brazil. Furthermore, the Tsylla Balbino Maternity ward serves the majority of low-income women in Salvador, who are almost exclusively blacks or mulattos²³. Among the newborns analyzed, only six (5 FSC and 1 FS) required special care, representing the symptomatic group that can develop severe clinical conditions and need early treatment. The 47 newborns identified as FAS displayed a racial distribution of 17.0% whites, 53.4% mulattos and 29.6% blacks. These variations of racial distribution of hemoglobin S among the newborns groups highlight the need for universal neonatal hemoglobinopathy screening in the Brazilian population. The comparison of hematological data among newborns with HbC and those with normal hemoglobin showed statistical significance for PVC, RBC, and MCHC due to increased blood viscosity, demonstrating the influence of HbC on hematological parameters^{8,9}.

The high frequencies of $\alpha_2^{3.7Kb}$ thalassemia (heterozygous 19.7%; homozygous 2.5%) are similar to those found in previous studies conducted in Brazilian populations¹¹. The presence of $\alpha_2^{3.7Kb}$ thalassemia was not associated

with premature delivery. However, van der Dijs et al.²⁵ speculated that the presence of α_2 thalassemia could decrease the capacity to extract oxygen from maternal circulation and could thus be a contributing factor to early delivery of newborn α_2 thalassemia carriers. Newborns with FAS and FAC pattern, such as the babies heterozygous for $\alpha_2^{3.7Kb}$ thalassemia, did not present symptoms of severe anemia. However, they still need genetic counseling²⁶.

We did not identify any newborns with $\alpha_2^{4.2Kb}$ thalassemia in our study, suggesting that this type of thalassemia is rare or absent in Bahia. The frequency of $\alpha_2^{3.7Kb}$ thalassemia among FAS newborns (20.9% of which were heterozygous and 4.7% of which were homozygous) could be beneficial for these S hemoglobin carriers and for the sickle cell anemia patients in Bahia. The α_2 thalassemia is considered a beneficial factor, since this condition is associated with less severe clinical manifestations among sickle cell disease carriers^{8,9}. It decreases MCHC and the rate of hemolysis, resulting in higher Hb, PCV, and RBC values. Therefore, some complications such as leg ulcers, renal pathology, and strokes become less frequent. However, the frequency of other complications such as osteonecrosis and retinopathy may be increased²⁷.

The comparison of hematological data among newborns with α -normal genes and those with $\alpha_2^{3.7Kb}$ thalassemia showed statistical differences in all hematological parameters analyzed, except for MCHC. The hematological analysis of newborns with FA and FAS hemoglobin groups and $\alpha_2^{3.7Kb}$ thalassemia carriers showed statistically significant differences in RBC and MCH; the comparison of the FA and FAC groups and $\alpha_2^{3.7Kb}$ thalassemia carriers

showed significant differences in MCH and MCHC. The hematological differences found between newborns with normal α -genes and $\alpha_2^{3.7Kb}$ thalassemia carriers could be intensified by the presence of variant hemoglobins. Furthermore, differentiation within the Bahian pediatric population needs to be developed in order to determine whether the presence of $\alpha_2^{3.7Kb}$ thalassemia could be responsible for intensifying the hematological pattern of ane-

mia, especially in child with iron deficiency, avoiding erroneous therapy.

In Bahia, the presence of hemoglobinopathies is a public health problem, and the early diagnosis of variant hemoglobin carriers provides an opportunity for counseling and early clinical follow-up of the child, both of which contribute to reduce child morbidity and mortality rates.

Resumo

Hemoglobinopatias são alterações hereditárias na molécula de hemoglobina com prevalência mundial elevada. O Brasil apresenta prevalência de 0,1 a 0,3% para recém-nascidos com anemia falciforme e frequência de 20,0 a 25,0% para a ocorrência de heterozigotos da talassemia α_2 entre indivíduos afro-descendentes. O presente estudo investigou a presença de hemoglobinas variantes e talassemia $\alpha_2^{3.7Kb}$ e $\alpha_2^{4.2Kb}$ em recém-nascidos de Salvador, Bahia, Brasil. Analisamos o sangue do cordão umbilical de 590 recém-nascidos, sendo 57 (9,8%) com padrão FAS; 36 (6,5%) FAC; um (0,2%) SF e cinco (0,9%) FSC. Cento e catorze (22,2%) apresentaram talassemia $\alpha_2^{3.7Kb}$, dos quais 101 (19,7%) foram heterozigotos e 13 (2,5%) homozigotos, mostrando significância estatística para os dados hematológicos entre recém-nascidos com genes α normais e portadores de talassemia $\alpha_2^{3.7Kb}$. A talassemia $\alpha_2^{4.2Kb}$ não foi encontrada. As frequências descritas neste trabalho confirmam que as hemoglobinopatias são um problema de Saúde Pública no Brasil, enfatizando a importância dos programas de triagem neonatal e aconselhamento genético.

Hemoglobinopatias; Anemia Falciforme; Talassemia; Recém-Nascido

Contributors

E. V. Adorno participated in the blood sample collection in the hospital, hemoglobin analysis, DNA extraction, molecular analysis, and drafting of the manuscript. F. D. Couto, J. F. Menezes and J. P. Moura Neto participated in the blood sample collection in the hospital, hemoglobin analysis, and DNA extraction. M. Rego participated in the statistical analysis. M. G. Reis participated in the interpretation of the results and drafting and revision of the manuscript. M. S. Gonçalves supervised the laboratory analysis, interpretation of the results, and drafting and revision of the manuscript.

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