

ALPHA THALASSEMIA FREQUENCY IN NEWBORN CHILDREN FROM PORTO ALEGRE, BRAZIL

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ABSTRACT

Blood samples from 599 Porto Alegre newborn children were screened by electrophoretic methods. The overall prevalence of Hb Bart's was 3.7%, with a higher (5.4%) frequency among Black, as compared to White (2.5%) babies. Twenty-one children showed Hb Bart's levels between 1 and 4% and one had 5.1% of this hemoglobin. This child had a reduced MCH and MCV, compatible with the thalassemic trait, and was classified as homozygous for α^+ thalassemia, while the infants with lower levels of Hb Bart's were considered heterozygotes for this condition. Maximum likelihood calculations indicated that this method detects only half of $-\alpha/\alpha\alpha$ individuals. Accordingly, the prevalence of haplotype $-\alpha$ was estimated as 6% in Black and as 2.5% in White children. Red cell counts and other hematological parameters, gestational age, weight and Apgar scores did not differ significantly between normal babies and carriers of Hb Bart's.

INTRODUCTION

Alpha-thalassemias are inherited disorders due to a partial (α^+ -thalassemia) or total (α^0 -thalassemia) reduction of α -globin synthesis. Deletion of one gene in α^+ and of both genes in α^0 -thalassemia are the most common mutations, and occur in different human populations (WHO, 1982).

Four clinical syndromes are recognized: the silent carrier with loss of a single α -gene ($-\alpha/\alpha\alpha$); α -thalassemia trait, in which two genes are missing, by loss of either both α -genes from a single chromosome ($--/\alpha\alpha$) or one gene from both chromosomes ($-\alpha/-\alpha$), Hb H disease, which is determined by the compound heterozygous state for the single and double deletion chromosomes ($--/-\alpha$), and hydrops fetalis, in which no α genes are present ($--/--$; Honig and Adams, 1986).

The newborn baby with α -thalassemia produces a relative excess of γ globin chains. Tetramers of γ chains (Hemoglobin Bart's) form and can be detected by electrophoresis. Detection of Hemoglobin Bart's in the neonatal period is therefore a useful method for α -thalassemia screening. But data about the prevalence of Hb Bart's in Brazil are scanty. Only two investigations have been performed thus far, and were restricted to the State of São Paulo (Zago *et al.* 1983; Sonati, 1987).

The present paper reports the prevalence of Hb Bart's in newborn children from the Southern Brazilian population of Porto Alegre and investigates the association of this phenotype with anemia and different parameters of fetal development.

SUBJECTS AND METHODS

Cord blood samples from 599 babies (60% White and 40% Black) were collected using EDTA as anticoagulant, at two maternities from Porto Alegre. From each child 10 ml of blood were obtained and separated into two vials; one aliquot was used for hemoglobin analysis and the other for the determination of standard hematological parameters. The samples were kept at 4°C until analysis.

Hemoglobin and hematocrit estimation, red cell count, MCV, MCH and MCHC indices were determined as described by Williams (1983). Hb H inclusion bodies were searched for after incubation of equal volumes of blood and 1% brilliant cresyl blue at 37°C for 2-3 hs (Lehmann and Huntsmann, 1974). These studies were conducted within a maximum of 24 hs after collection.

Hemoglobin electrophoresis was performed on cellulose acetate strips, as described by Serjeant *et al.* (1974). Samples which were found to have a fast-moving band were further subjected to electrophoresis on the same support medium, using 0.1 M phosphate buffer, pH 6.5, for confirmation of the presence of Hb Bart's. Quantitative determination of this hemoglobin was achieved by measurement of the optical density at 415 nm, after elution from the cellulose acetate strips (Weatherall, 1983).

Samples with bands at the Hb S or Hb C position were further analysed by electrophoresis on agar gels according to the method of Serjeant *et al.* (1974).

Birth weight, Apgar score, gestational age, and presence or absence of congenital malformations were obtained from the hospital files. Afterwards, data about the nationality of grandparents, socioeconomic level, previous reproductive and morbid histories, and parents' consanguinity were obtained through an interview with the mother.

RESULTS

As indicated in Table I, Hemoglobin Bart's was detected in 22 of 599 newborn infants, giving an overall incidence of 3.7%. As expected the prevalence of this hemoglobin was higher in Black (5.4%) as compared to White babies (2.5%). The

levels observed ranged from 1.0 to 5.1%; 21 children had values between 1 and 4%, while one Black baby showed the highest figure of 5.1% of Hb Bart's. Hb H inclusion bodies were not detected in these infants.

Table I - Frequency of newborn children with Hb Bart's in a sample from Porto Alegre.

Hb Bart's (%)	Whites		Blacks		Total	
	N	%	N	%	N	%
Not detected	351	97.5	226	94.6	577	96.3
1.0 - 4.0	9	2.5	12	5.0	21	3.5
> 5.0	-	-	1	0.4	1	0.2
Total	360	100.0	239	100.0	599	100.0

The hematological parameters observed in this sample are shown in Table II. The infant with 5.1% of Hb Bart's had a lower MCV and MCH when compared to the observed means of the other two groups of children. This reduction suggests that this individual has only two α genes. In the majority of cases of African origin, the α thalassemias are due to a deletion of 3.7 kb of DNA ($-\alpha^{3.7}$ "rightward deletion"; Dozy *et al.*, 1979). Rarely this syndrome, in this ethnic group, may be a result of chromosomes that have lost both α genes ($--$) or of point mutations (Embury *et al.*, 1980; Steinberg and Embury, 1986). Therefore, this child is probably homozygote for the α^+ determinant ($-\alpha/-\alpha$), and those with 1 to 4% of Hb Bart's would be heterozygotes for this condition ($-\alpha/\alpha\alpha$).

Table II - Hematological parameters in newborn children with and without Hb Bart's.

Hematological parameter	Babies without Hb Bart's (N = 531)		Babies with 1-4% Hb Bart's (N = 21)		Child with 5.1% Hb Bart's
	Mean	SD	Mean	SD	
Hematocrit (%)	48.54	4.95	47.62	6.82	50.0
Hemoglobin (g/dl)	15.83	1.76	15.40	2.10	16.1
Red Cell Number ($\times 10^6/\text{mm}^3$)	4.77	0.59	4.86	0.69	5.5
MCV (fl)	102.18	9.88	98.04	11.44	91.0
MCH (pg)*	33.21	3.43	31.52	3.44	29.0
MCHC (%)	32.62	1.90	32.69	1.48	32.2

* Significant difference between the means of the two groups ($P < 0.05$).

Assuming that this genotype is segregating in this population, random mating, and that a proportion of the heterozygotes would not be detected by the methods used in the present study (Higgs *et al.*, 1982) it is possible to devise a model to calculate the frequency of the $-\alpha$ haplotype and of the fraction of undetected heterozygotes. The results obtained are summarized in Table III. Using an unpublished computer program developed by P.H. Cabello and H. Krieger we estimated this fraction to be 51%. Accordingly, the frequencies of $-\alpha/\alpha\alpha$ and $-\alpha/-\alpha$ should be doubled, and the $-\alpha$ haplotype frequency should be estimated as being respectively 6% and 2.5% in the Black and White populations of Porto Alegre.

Two Hb AS and two Hb AC individuals were found among the White children, the corresponding figures for the Black being three Hb AS and four Hb AC. Of these, only one Hb AS black child was concomitantly a presumably $-\alpha/\alpha\alpha$ heterozygote.

Table III - Estimation of the fraction of undetected heterozygotes in the present study.

Hb Bart's level	Probable genotype	Blacks			Whites		
		N	Observed frequency	Expected frequency	N	Observed frequency	Expected frequency
Not detected	$\alpha\alpha/\alpha\alpha$	226	0.9456	0.8888	351	0.9750	0.9506
1 - 4%	$-\alpha/\alpha\alpha$	12	0.0502	0.1079	9	0.0250	0.0488
> 5%	$-\alpha/-\alpha$	1	0.0042	0.0039	-	-	0.0006

Maximum likelihood model used for this estimation, x being the fraction of undetected heterozygotes: $\alpha\alpha/\alpha\alpha: p^2 + 2pqx$; $-\alpha/\alpha\alpha: 2pq(1-x)$; $-\alpha/-\alpha: q^2$.

Haplotype frequencies: Blacks, $\alpha\alpha: 0.94276 \pm 0.0145$; $-\alpha: 0.05724$; Whites, $\alpha\alpha: 0.97515 \pm 0.00839$; $-\alpha: 0.02485$. Fraction of undetected heterozygotes, estimated by pooling the two samples: 0.51288 ± 0.10415 .

The hematocrit, hemoglobin concentration and red cell number means did not differ significantly between putative $\alpha\alpha/\alpha\alpha$ and $-\alpha/\alpha\alpha$ subjects, but there was a significant lowering of MCH (33 vs 31.5 pg.) and a somewhat (non-significant) decrease of MCV (102 vs 98 fl) among the latter (Table II).

No effect of the presence of Hb Bart's on the birth conditions, as evaluated by Apgar scores, weight and gestational age of the babies could be detected. The other information obtained assured that the samples were representative of the city's population, as assessed by previous investigations (see, for instance, Araújo and Salzano, 1974, 1975).

DISCUSSION

The true prevalence of α thalassemia is not represented by that of Hb Bart's. However, most studies certainly detected levels above 2%; therefore, they were capable of picking up α^+ thalassemia homozygotes, allowing the estimation of this prevalence in a given population (Higgs *et al.*, 1980; Kanavakis *et al.*, 1986; Lallemand *et al.*, 1986).

Table IV shows the large variation in the proportion of newborn children with elevated Hb Bart's levels among Blacks, both in Africa and in the New World. This frequency ranged from 30% in the series investigated by Horton *et al.* (1962) in the United States, to 1.7% in Senegal (Oudart *et al.*, 1968). In Brazil Sonati (1987) observed 11.9% of Hb Bart's carriers among Black children from Campinas, São Paulo, while in the present series we detected only 5.4% of Black babies with this hemoglobin.

Part of this apparent variability in the frequency of α thalassemia could be due to the sensitivity of the techniques used in the detection of the lower levels of Hb Bart's found in α^+ thalassemia heterozygotes (Higgs *et al.*, 1982). But true genetic differences among Black populations could also account for this variation. The different values observed between the two Black Brazilian series could also be explained by different degrees of admixture. The children investigated in Campinas were born mainly (58%) from Black mothers without clear evidences of admixture, while the majority (81%) of the Black sample from Porto Alegre was classified as Mulatto.

Restriction endonuclease mapping studies of the α globin gene cluster from individuals living in Benin, Central African Republic and Senegal showed that the prevalence of the $-\alpha$ haplotype ranged from 10% to 14% in these populations (Pagnier *et al.*, 1984). Through the Hb Bart's level we have estimated the prevalence of this haplotype in Porto Alegre Blacks as being 6%. This figure fits well that expected, considering that the degree of White admixture calculated for this population is of the order of 50% to 65% (Franco *et al.*, 1982).

In the present paper we report for the first time the prevalence of Hb Bart's carriers in a White Brazilian population. The proportion observed (2.5%) is very close to that obtained by Velati *et al.* (1986) in North Italy (3%; Table IV). The identification of Italian ancestry among the carriers of the present study, however, was possible in two cases only. One child had a grandfather born in Italy, and the other had an Italian surname. Two hypotheses could explain this observation: (a) Loss of surnames of Mediterranean origin; and (b) Black admixture among these individuals. As was already mentioned, we observed two putative White babies with Hb AS and two with Hb AC, clearly indicating African admixture. Franco *et al.* (1982), using these and five other markers, estimated in 8% the degree of Black admixture among the Porto Alegre Whites. Therefore, these two hypotheses are not mutually exclusive, and it is

possible that this population shares α thalassemia genes from both Mediterranean and African origins.

Table IV - Frequency of Hemoglobin Bart's carriers in various populations.

Countries and populations	Sample size	Frequency (%)	References
<i>Africa</i>			
South Africa	1207	3.3	Rousseau <i>et al.</i> (1985)
P.R. Congo	146	23.3	Lallemant <i>et al.</i> (1986)
Zaire	636	17.9	Van Baelen <i>et al.</i> (1969)
Nigeria	700	6.0	Kulkarni and Jekeme (1986)
Senegal	345	1.7	Oudart <i>et al.</i> (1968)
Sudan	64	9.3	Vella and Verzin (1963)
Tanzania	325	11.0	Nhonoli <i>et al.</i> (1979)
<i>Europe</i>			
Spain	2003	0.2	Calero <i>et al.</i> (1982)
Greece	227	2.2	Kanavakis <i>et al.</i> (1986)
Italy (Sardinia)	2291	12.9	Galanello <i>et al.</i> (1980)
Italy (Milan)	4730	3.0	Velati <i>et al.</i> (1986)
<i>America</i>			
USA (Blacks)	900	2.1	Weatherall (1963)
USA (Blacks)	693	15.0	Friedman <i>et al.</i> (1974)
USA (Blacks)	300	30.0	Horton <i>et al.</i> (1962)
USA (Blacks)	9000	5.0	Schneider <i>et al.</i> (1974)
USA (Whites)	7000	1.0	Schneider <i>et al.</i> (1974)
Cuba	2363	4.3	Martinez and Cañizares (1982)
Guadeloupe	6545	3.5	Lallemant <i>et al.</i> (1986)
Jamaica	2191	7.0	Higgs <i>et al.</i> (1980)
Brazil (Ribeirão Preto, mixed)	606	0.7	Zago <i>et al.</i> (1983)
Brazil (Campinas, Blacks)	320	11.9	Sonati (1987)
Brazil (Porto Alegre, Blacks)	239	5.4	Present study
Brazil (Porto Alegre, Whites)	360	2.5	Present study

The main advantage of detecting α -thalassemia in Blacks is to distinguish them from iron deficiency patients, avoiding unwarranted treatment and testing. The $-\alpha/-\alpha$ genotype is associated with microcytic erythrocytes and sometimes with mild

anemia (Steinberg and Embury, 1986). The only homozygote child for α^+ thalassemia observed in this study was not anemic, but had a marked reduction in his MCV and MCH indices (Table II).

Lallemant *et al.* (1986) noted in a group of Congolese Hb Bart's carriers a significantly lower weight at birth, but could not explain this observation. They suggested new studies to confirm this finding. In the present series the birth weight and gestational age of children with Hb Bart's did not differ from those observed in normal infants. Results in this same direction were also obtained by Van Baelen *et al.* (1969) and Pembrey *et al.* (1975) for babies from Zaire and Saudi Arabia respectively.

The Apgar scores, which measure the vital conditions at birth, also did not show any difference between Hb Bart's carriers and normal babies. To our knowledge, the investigation of this index on Hb Bart's children had never been reported up to now.

These results suggest that the absence of one or two alpha genes has no detrimental effects on pregnancy outcome or the vital conditions of newborn children, unlike the deletion of four alpha genes, which produces fetal hydrops and maternal toxemia (Maccioni *et al.*, 1986).

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RESUMO

Foram estudadas 599 amostras de sangue do cordão umbilical de recém-nascidos de Porto Alegre, através de métodos eletroforéticos. A prevalência de Hb Bart's foi de 3,7%, com uma frequência maior em bebês negros (5,4%) do que em brancos (2,5%). Vinte e um recém-nascidos apresentaram níveis de Hb Bart's entre 1 e 4%, e um 5,1% desta hemoglobina. Esta criança apresentou uma redução no VCM e HCM compatíveis com o traço talassêmico, sendo, portanto, classificada como homocigota para a talassemia α^+ , enquanto os recém-nascidos com níveis mais baixos de Hb Bart's foram considerados heterocigotos para esta condição. Cálculos de máxima verossimilhança indicaram que este método detecta somente a metade dos indivíduos $-\alpha/\alpha$. Sendo assim, a prevalência do haplótipo $-\alpha$ foi estimada em 6% e 2,5% entre negróides e caucasoídes, respectivamente. O número de eritrócitos e outros parâmetros

hematológicos, a idade gestacional, o peso e os índices de Apgar não diferiram significativamente entre os bebês normais e os portadores da Hb Bart's.

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