High prevalence of α-thalassemia among individuals with microcytosis and hypochromia without anemia

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Abstract

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In order to determine the contribution of α-thalassemia to microcytosis and hypochromia, 339 adult outpatients seen at Unicamp University Hospital (with the exception of the Clinical Hematology outpatient clinics), who showed normal hemoglobin (Hb) levels and reduced mean corpuscular volume and mean corpuscular hemoglobin, were analyzed. Ninety-eight were Blacks (28.9%) and 241 were Caucasians (71.1%). In all cases, Hb A_2 and F levels were either normal or low. The most common deletional and nondeletional forms of α-thalassemia [$-\alpha^{3.7}$, $-\alpha^{4.2}$, $--^{MED}$, $-(\alpha)^{20.5}$, $\alpha^{HphI}\alpha$, $\alpha^{NcoI}\alpha$, $\alpha\alpha^{NcoI}$ and α^{TSAUDI}] were investigated by PCR and restriction enzyme analyses. A total of 169 individuals (49.9%) presented α-thalassemia: 145 (42.8%) were heterozygous for the $-\alpha^{3.7}$ deletion ($-\alpha^{3.7}/\alpha\alpha$) and 18 (5.3%) homozygous ($-\alpha^{3.7}/-\alpha^{3.7}$), 5 (1.5%) were heterozygous for the nondeletional form $\alpha^{HphI}\alpha$ ($\alpha^{HphI}\alpha/\alpha\alpha$), and 1 (0.3%) was a $--^{MED}$ carrier ($--^{MED}/\alpha\alpha$). Among the Blacks, 56 (57.1%) showed the $-\alpha^{3.7}/\alpha\alpha$ genotype, whereas 12 (12.2%) were $-\alpha^{3.7}/-\alpha^{3.7}$ and 1 (1.0%) was an $\alpha^{HphI}\alpha$ carrier; among the Caucasians, 89 (36.9%) were $-\alpha^{3.7}/\alpha\alpha$, 6 (2.5%) had the $-\alpha^{3.7}/-\alpha^{3.7}$ genotype, 4 (1.7%) presented the nondeletional form ($\alpha^{HphI}\alpha/\alpha\alpha$), and 1 (0.4%) was a $--^{MED}$ carrier. These results demonstrate that α-thalassemia, mainly through the $-\alpha^{3.7}$ deletion, is an important cause of microcytosis and hypochromia in individuals without anemia. These data are of clinical relevance since these hematological alterations are often interpreted as indicators of iron deficiency.

Introduction

Microcytosis and hypochromia result from deficient hemoglobin (Hb) synthesis in erythroid cells, causing a reduction in both mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) of red blood cells. Without the increase in Hb A_2 levels, these hematological alterations may be due to α-thalassemia, iron deficiency or, occasionally, chronic disease anemia (1,2).

α-Thalassemia is the most common genetic disorder of Hb synthesis in the world, with gene frequencies varying between 1% and 98% throughout the tropics and subtropics, where *Plasmodium falciparum* is or has

been endemic, or in populations which received people from these areas through immigration (3-5). It results from an imbalance in α-globin chain production, which can be reduced (α^+-thalassemia) or completely abolished (α^0-thalassemia). Most commonly α-thalassemia results from deletion of one (-α) or both (--) of the duplicated α genes ($\alpha\alpha$) on chromosome 16p13.3. Less frequently, it is caused by small deletions or point mutations (so-called nondeletional α-thalassemia) involving the predominantly expressed α_2 gene ($\alpha^T\alpha$) or rarely the α_1 gene ($\alpha\alpha^T$), or yet by deletions outside the α cluster which leave the structural genes intact but without expression (3,6).

The α-thalassemia phenotypes range from mild microcytic hypochromic anemia to a hemolytic anemia of variable severity characterized by the presence of Hb H in the case of loss of three functional α genes, or to the hydrops fetalis syndrome, characterized by severe intrauterine anemia and fetal or perinatal death due to the loss of all four α globin genes (1-3). The most common cause of α-thalassemia is a deletion of 3.7 kb of DNA originated by homologous recombination between misaligned chromosomes, which affects both α genes in *cis* and results in a unique hybrid gene ($\alpha_2\alpha_1$) (-$\alpha^{3.7}$ deletion). The hematological alterations caused by this deletion, also known as rightward α^+-thalassemia, can be very mild, if not silent (7-9). It is most prevalent in the African and Mediterranean regions. Other relatively frequent causes of α-thalassemia are the -$\alpha^{4.2}$ deletion (leftward α^+-thalassemia) found in Asian and Mediterranean populations, the --[MED] and -(α)$^{20.5}$ deletions, common causes of α^0-thalassemia in the Mediterranean region, and the --[SEA] deletion, found with high frequency in Southeast Asia (7). Among the most common nondeletional forms, $\alpha^{HphI}\alpha$ is a pentanucleotide deletion in the splice donor site of IVS-I which abolishes a *Hph*I site in the α_2 gene; $\alpha^{NcoI}\alpha$ and the $\alpha\alpha^{NcoI}$ are caused by base substitutions in the translational initia-

tion codon ATG in the α_2 or in the α_1 gene, respectively, which presumably completely abolish translation and can be recognized by the loss of the *Nco*I site present in the initiation codon, and a third mutation, $\alpha^{TSAUDI}\alpha$, caused by a base substitution in the highly conserved polyadenylation signal sequence AATAAA, which must prevent endonucleolytic cleavage and poly A addition to the 3' end of mRNAs (7). They are all encountered in Mediterranean populations. In Brazil, the -$\alpha^{3.7}$ deletion has been frequently found in the Black population (10,11), and the --[MED] and -(α)$^{20.5}$ deletions have sporadically been described (12,13). One family with the nondeletional form $\alpha^{HphI}\alpha$ has been recently reported (13).

Individuals with microcytosis and hypochromia, without anemia, have been detected in clinical laboratories. In order to determine the contribution of α-thalassemia to such cases, we analyzed 339 adult individuals, followed as outpatients at Unicamp University Hospital (with the exception of the Clinical Hematology outpatient clinics), who showed normal Hb levels together with reduced MCV and MCH and normal or decreased Hb A_2 (and F).

Material and Methods

Subjects

A total of 339 adult individuals (age >14 years), followed as outpatients at Unicamp University Hospital, Campinas, State of São Paulo, Southeast Brazil, with the exception of the Clinical Hematology outpatient clinics, were analyzed. The subjects presented normal Hb levels (Hb $\geq$14 g/dl for men and $\geq$12 g/dl for women) and reduced MCV ($\leq$80 fl) and MCH ($\leq$27 pg). Ninety-eight individuals were Blacks (28.9%), including Mulattoes and Negroes, and 241 were Caucasians (71.1%). In all cases, Hb A_2 and F levels were normal or decreased (<3.6 and <2.0%, respectively).

The patients studied here formally consented to be investigated for the presence of α-thalassemia.

Methods

Red blood cell indices were electronically determined (Cell Dyn 3500, Abbott Laboratories, Chicago, IL, USA), and Hb analyses were carried out according to Weatherall and Clegg (1).

DNA samples were obtained from peripheral blood leukocytes by organic extraction. With the exception of the --^{SEA} deletion, all the other deletional and nondeletional forms of α-thalassemia mentioned above were investigated by PCR-based methods. Rightward deletion (-α$^{3.7}$) was detected by the method of Dodé et al. (14); --^{MED} and -(α)$^{20.5}$ were investigated as described by Bowden et al. (15), and the -α$^{4.2}$ leftward deletion was screened by the method of Oron-Karni et al. (16). Nondeletional forms were investigated according to Hall et al. (17) using the corresponding restriction enzymes (*Hph*I and *Nco*I) and a specific nested PCR for α^{TSAUDI} (15).

Serum ferritin levels were determined by an automated chemoluminescent immuno-enzymatic method (Immulite, Diagnostic Products Co., Los Angeles, CA, USA) for all α-thalassemic cases to make sure that microcytosis and hypochromia were not due to concomitant iron deficiency.

Results

The results are summarized in Table 1. Among the 339 individuals studied, 169 (49.9%) presented α-thalassemia: 145 (42.8%) were heterozygous for the -α$^{3.7}$ deletion (-α$^{3.7}$/αα), 18 (5.3%) were homozygous (-α$^{3.7}$/-α$^{3.7}$), 5 (1.5%) showed the nondeletional form α^{HphI}α/αα, and 1 (0.3%) was a --^{MED} carrier (--^{MED}/αα). Among the 98 Blacks, 56 (57.1%) showed the -α$^{3.7}$/αα genotype, while 12 (12.2%) were -α$^{3.7}$/-α$^{3.7}$

and 1 (1.0%) was α^{HphI}α/αα; among the Caucasians, 89 (36.9%) were -α$^{3.7}$/αα, 6 (2.5%) had the -α$^{3.7}$/-α$^{3.7}$ genotype and 4 (1.7%) were α^{HphI}α/αα. The --^{MED} carrier belonged to this racial group (0.4%).

The serum ferritin levels determined for the α-thalassemia cases were all above the lower normal limits (9 ng/ml for women and 19 ng/ml for men).

Discussion

The present results demonstrated that α-thalassemia, mainly through the -α$^{3.7}$ deletion, is an important cause of microcytosis and hypochromia in individuals without anemia, indicating that non-anemic Brazilian Blacks, with low MCV and MCH, have a 70.4% chance of carrying α-thalassemia, whereas this chance is of 41.5% among Brazilian Caucasians, i.e., still high. The -α$^{3.7}$ deletion is known to occur at significant frequencies in Black populations (7). Since in Brazil there is an elevated degree of miscegenation, it seems that even in the non-Black population its prevalence is also high.

The present data are of clinical relevance, since microcytosis and hypochromia are often interpreted as indicators of iron deficiency and patients may be mistreated with oral iron therapy (18). In about 50% of the cases analyzed here, the cause of these hematological alterations was α-thalassemia. It is possible that this proportion is still a little higher, because many silent mutations

Table 1. Genotypes found among 339 individuals with microcytosis and hypochromia without anemia.

	Caucasians	Blacks	Total
αα/αα	141/241 (58.5%)	29/98 (29.7%)	170 (50.1%)
-α$^{3.7}$/αα	89/241 (36.9%)	56/98 (57.1%)	145 (42.8%)
-α$^{3.7}$/-α$^{3.7}$	6/241 (2.5%)	12/98 (12.2%)	18 (5.3%)
α^{HphI}α/αα	4/241 (1.7%)	1/98 (1.0%)	5 (1.5%)
--^{MED}/αα	1/241 (0.4%)	-	1 (0.3%)
Total	241 (71.1%)	98 (28.9%)	339 (100%)

causing α-thalassemia and other not so common deletions may not have been detected.

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