



Co-Inheritance of alpha-thalassemia and sickle cell disease in a cohort of Angolan pediatric patients

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Abstract

The aim of this study was to explore the association between alpha-thalassemia, fetal hemoglobin, hematological indices, and clinical adverse events in Angolan sickle cell disease pediatric patients. A total of 200 sickle cell disease (SCD) children were sampled in Luanda and Caxito. A venous blood sample was collected and used for hematological analyses, fetal hemoglobin quantification, and genotyping of 3.7 kb alpha-thalassemia deletion by GAP-PCR. The frequency of the 3.7 kb alpha-thalassemia deletion in homozygosity was 12.5% and in heterozygosity was 55.0%. An increase in alpha-thalassemia frequency was observed in children older than 5 years old (11.7% vs. 13.00%). Furthermore, 3.7 kb alpha-thalassemia deletion homozygotes had a significantly higher age of the first manifestation, lower number of blood transfusions by year, higher hemoglobin, lower mean corpuscular volume, mean corpuscular hemoglobin, and lower hemolytic rate observed by a lower number of reticulocytes count. There were no differences in fetal hemoglobin between the three genotypes. Moreover, the number of stroke events, osteomyelitis, splenomegaly, splenectomy, and hepatomegaly were lower when alpha-thalassemia was co-inherited. For the first time in Angolan population, the effect of alpha-thalassemia deletion in sickle cell disease was analyzed and results reinforce that this trait influences the hematological and clinical aspects and produces a milder phenotype.

Keywords 3.7 kb alpha-thalassemia deletion · Angola · Fetal hemoglobin · Sickle cell disease

Introduction

Sickle cell disease (SCD) is a recessive hereditary disease and a major global health problem, affecting over 300.000 newborn infants each year and it is estimated that 75% of these births occur in Sub-Saharan Africa [1, 2]. This widely neglected disease has a mortality rate of around 50–90% among undiagnosed SCD children under the age of 5, which may contribute up to 5% of child mortality on the African continent [3, 4]. Fortunately, after diagnosis, the mortality can be greatly reduced, and an increase in life expectancy is observed. A study developed in Angola indicates that early diagnosis and basic preventive treatments are highly cost-effective regardless of which mortality rate is assumed [5].

Even though SCD is a monogenic disease, the clinical manifestations are very heterogeneous due to environmental and genetic factors, in particular, the co-inheritance of alpha-thalassemia and an innate ability to produce fetal hemoglobin, are two major modifiers that have a substantial impact on disease pathophysiology [6]. A great proportion

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of African descendants with SCD have this co-inheritance of alpha-thalassemia due to the common 3.7 kb deletional variant [7]. These patients have a lesser degree of hemolysis, and consequently fewer complications, due to the reduction of S hemoglobin (HbS) intracellular concentration and the reduction of HbS polymerization and consequently red cell sickling [7, 8]. However, while there is a protective effect against complications associated with hemolysis, the increased hematocrit and blood viscosity may have a consequence in the increase of other complications associated with microvascular occlusion (such as increased acute pain, acute chest syndrome, osteonecrosis, and retinopathy) [9, 10].

Most of the information concerning the association between alpha-thalassemia and SCD comes from African descendent patients living in the Northern Hemisphere, and there is a concerning lack of data on this co-inheritance in Africa and a total absence of information in Angola.

It is estimated that up to 5% of the world's population carries at least one alpha-thalassemia variant, but in some tropical countries, the carrier frequency may be as high as 80% of the population [11, 12]. Due to this geographic variability, there is a growing demand for further studies among different ethnic cohorts to fully understand the impact of this co-inheritance on disease severity.

In that sense, this study aimed to explore the association between alpha-thalassemia, fetal hemoglobin, hematological indices, and clinical serious events in Angolan sickle cell disease hydroxyurea-naïve pediatric patients. Moreover, we intend to determine the prevalence of 3.7 kb alpha-thalassemia deletion in this population.

Methods

Patients and samples

This cross-sectional study is part of a large study in an Angolan SCD cohort conducted in the Hospital Pediátrico David Bernardino in Luanda and Hospital Geral do Bengo in Caxito. Sampling was performed between April and August 2019. A total of 200 sickle cell disease children were selected, after guardian written informed consent. None of them was treated with hydroxyurea or transfusion in the last 3 months.

About 4 mL of whole blood samples were collected in EDTA tubes from each participant in the context of the routine medical follow-up of the patients and used for hematological analyses, study of hemoglobin fractions and DNA genotyping. The following hematological parameters were measured: hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), white blood cells count, neutrophils count, platelet count, reticulocyte count

using the XT-2000i Hematology Analyzer (Sysmex Corporation, Kobe, Japan). The different hemoglobin fractions were quantified by high-performance liquid chromatography using a Variant II device (Biorad, Hercules, CA).

Molecular study

DNA isolation was done by the Qiagen Blood Mini Kit (Cat No./ID 51106, Qiagen, USA), and the 3.7 kb alpha-thalassemia deletion was studied by GAP-PCR according to published literature [13]. In short, two PCR mixtures were prepared in two PCR reactions with three separate primers (one of the primers is used in both reactions). This method can detect the hybrid fragment which exists with deletion of 3.7 kb.

Statistical analysis

Mean values, standard deviation, and frequency distributions were performed to estimate the hematological, clinical, and genetic data. Hardy–Weinberg equilibrium was determined using GENEPOP [14]. ANOVA, non-parametric tests, and χ^2 tests were applied to compare means, medians, or frequencies between the three alpha-thalassemia genotypes using IBM® SPSS® Statistics 25 software (Armonk, NY, USA). P-value < 0.05 was considered statistically significant.

This study was approved by the Ethical Committee of the Ministry of Health of Angola (CE. N° 040/2018) and the Ethical Committee of Escola Superior de Tecnologia da Saúde de Lisboa (CE-ESTeSL-N°. 43-2018). Informed consent was obtained and signed by the caregivers of children. All the children's consultation and follow-up were performed by the project team freely.

Results

Molecular characteristics of the sample

A total of 200 SCD children were studied (103 females and 97 males, representing 51.5% and 48.5% of the sample, respectively) with an average age of 6.6 years old, ranging from 3 to 12 years old. The observed frequency of homozygotes for 3.7 kb alpha-thalassemia deletion was 12.5% (Table 1) being the deletion allelic frequency of 40% in this sample. We observed a slight deviation from Hardy–Weinberg equilibrium ($\chi^2 = 4.25$, $p = 0.039$) with a decrease in the wild-type genotype (32.5%) and an increase in the 3.7 kb alpha-thalassemia deletion heterozygotes (55.0%) and homozygotes (12.5%).

Additionally, an increase in 3.7 kb alpha-thalassemia deletion frequency was observed in children older than 5 years old (genotype frequencies 11.7% vs. 13.00%, allelic

Table 1 Frequency of 3.7 kb alpha-thalassemia deletion in total sampled population and subdivided by age group (under 5 and over 5 years old)

3.7 kb alpha-thalassemia deletion genotype	Total sampled population (N=200)	Under 5 years old (N=77; 40%)	Over 5 years old (N=123; 60%)
$\alpha\alpha/\alpha\alpha$	65 (32.50%)	26 (33.80%)	39 (31.70%)
$\alpha\alpha/-\alpha3.7$	110 (55.00%)	42 (54.50%)	68 (55.30%)
$-\alpha3.7/-\alpha3.7$	25 (12.50%)	9 (11.70%)	16 (13.00%)

frequencies 38.95% vs. 40.65%). Although not statistically significant, this trend suggests that the co-inheritance of alpha-thalassemia may improve the survival of SCD patients promoting less morbidity when comparing to SCD patients with the alpha-globin wild-type genotype. However, the age range is very small (3 to 12 years) to get any conclusions in this cohort.

Effect of 3.7 kb alpha-thalassemia deletion on SCD phenotype

The patients homozygotes for the deletion tend to have higher age when the first manifestation (6 vs. 11 months; $p=0.053$), and a significantly lower number of blood transfusions per year (0.48 vs. 0.18; $p=0.031$), higher hemoglobin level (7.24 vs. 7.78 g/dL; $p=0.044$), lower MCV (81.75 vs. 62.73 fL; $p<0.001$), MCH (27.28 vs. 20.25 pg; $p<0.001$) and number of reticulocytes (11.62 vs. 6.46 $10^3/L$; $p<0.001$) (Table 2). There were no significant differences in fetal hemoglobin between the three groups of genotypes. Although it was not significant, we observed lower fetal hemoglobin values in 3.7 kb alpha-thalassemia deletion homozygotes as other studies have previously observed [8]. Moreover, the number of cases of stroke, osteomyelitis, splenomegaly, splenectomy, and hepatomegaly were lower in the presence of the deletion. It is noteworthy that in the subgroup of patients presenting alpha-thalassemia in the homozygous state, there were no events of stroke, osteomyelitis and splenectomy (Table 2).

Discussion

The protective effect of the alpha-thalassemia deletion in sickle cell disease patients is still controversial [15, 16], demonstrating the need for further studies in specific populations where the two pathologies reach high prevalence, such as the case of African populations. The Angolan population, in the southwest of Africa, has a high frequency of SCD as demonstrated in a recent screening performed in 36,453 newborn children where an incidence of 22,54% of sickle hemoglobin was observed with 1.5% of homozygous SS and 21,03% of heterozygous AS [5] and similar results obtained in another study performed in Bengo Province [17].

However, the prevalence of alpha-thalassemia in this population, as well as the role of its co-inheritance in sickle cell disease morbidity, is still unknown, which led to the development of the present study.

In the present study, 12.5% of SCD patients co-inherited the 3.7 kb alpha-thalassemia deletion in homozygosity. This homozygous frequency is similar to the one observed in Saudi Arabia (14,0%) [8] but higher than the results observed in Brazil (1,4%) [18], Georgia-USA (1.7%) [19], Senegal (2.3%) [16], Cameroon (6.8%) [20] and France (8.6%) [15] and lower than the one observed in Democratic Republic of Congo (25.5%) [21] and in New Delhi, India (38.9%) [22]. Despite all this variability, caution should be taken when comparing these frequencies across populations due to different sampling methods (e.g. more severe or less severe patients were included in different studies) and according to some authors, the advantageous effect of the alpha-thalassemia deletion in SCD patients could be associated with geographic altitude, patient age, and malaria endemicity in the region [23].

Some studies have reported a differential survival of SCD patients with co-inheritance of alpha-thalassemia deletion, with higher mortality for the one who has the normal alpha-genotype or for heterozygous [20]. In our study, the patient's age range between 3 and 12 years old and a slight increase in 3.7 kb alpha-thalassemia deletion frequency was observed in children older than 5 years old, which is in accordance with this hypothesis. Further studies, including SCD adults, should be performed in Angola in order to confirm this possible differential survival.

On the other hand, regarding morbidity, our results have shown a notorious benefit of the co-inheritance of alpha-thalassemia with the SCD, specifically attending to some clinical adverse events such as stroke and osteomyelitis. It is noteworthy that, unlike the other groups, the group presenting homozygosity for alpha-thalassemia never experienced stroke or osteomyelitis events. In agreement, this positive effect has been already reported by other authors [24]. Also, this group has a significantly higher age of the first manifestation (6 months vs. 11 months) and this consequent late disease onset was also already observed in other African populations with the same condition [20].

The present study confirms that 3.7 kb alpha-thalassemia deletion is associated with improved hematological

Table 2 Clinical adverse events and hematological phenotype subdivided by the three alpha-globin genotypes

Data	$\alpha\alpha/\alpha\alpha$		$\alpha\alpha/\alpha3.7$		$-\alpha3.7/\alpha3.7$		p
	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	
Age 1st manifestation months (median)	6	2–72	9	2–72	11	3–96	0.053 ^a
No of transfusions year	0.480 (0.679)	0.00–3.75	0.350 (0.409)	0.00–2.43	0.179 (0.170)	0.00–0.50	0.031^b
No of hospitalizations year	0.558 (0.601)	0.00–3.14	0.445 (0.447)	0.00–2.50	0.347 (0.327)	0.00–1.20	0.143 ^b
Stroke: yes (N, %)	5 (7.7)	–	1 (0.9)	–	0 (0.0)	–	na
Osteomyelitis: yes (N, %)	5 (7.7)	–	1 (0.9)	–	0 (0.0)	–	na
Splenomegaly: yes (N, %)	6 (9.2)	–	12 (10.9)	–	3 (12.0)	–	0.951 ^c
Splenectomy: yes (N, %)	2 (3.1)	–	1 (0.9)	–	0 (0.0)	–	na
Hepatomegaly: yes (N, %)	12 (18.5)	–	22 (20.0)	–	1 (4.0)	–	0.158 ^c
Hemoglobin (g/dL)	7.246 (0.987)	5.10–9.30	7.236 (0.981)	4.70–10.50	7.788 (1.237)	5.10–10.50	0.044^b
MCV (fL)	81.755 (5.387)	69.90–93.20	76.216 (6.745)	57.60–107.10	62.736 (5.761)	55.60–81.50	0.000^b
MCH (pg)	27.289 (2.054)	21.60–31.20	25.055 (2.248)	19.30–30.40	20.252 (1.957)	18.40–28.60	0.000^b
White blood cells count (10^3 L)	15.574 (4.705)	7.86–35.47	14.628 (4.886)	6.15–35.11	13.219 (7.519)	4.39–44.79	0.150 ^b
Neutrophils count (10^3 L)	6.715 (2.910)	2.49–15.97	6.680 (3.640)	1.06–25.52	6.321 (3.032)	1.98–12.43	0.887 ^b
Platelet count (10^3 L)	441.369 (164.644)	162.00–1004.00	427.200 (209.119)	68.00–1710.00	342.600 (188.165)	114.00–922.00	0.087 ^b
Reticulocyte count (10^3 L)	11.623 (4.513)	3.92–26.68	10.669 (5.364)	2.94–39.37	6.462 (4.457)	0.67–17.08	0.000^b
Fetal hemoglobin (%)	6.347 (4.539)	0.80–23.60	5.634 (4.000)	0.00–23.80	4.917 (3.468)	1.20–12.50	0.304 ^b

Bold indicates the significant values ($p < 0.05$)

na test not performed due to empty cells, SD standard deviation

^aKruskal–Wallis non parametric test

^bANOVA

^cPearson χ^2

indices, reduced hemolytic rate and reduced anemia in these patients. Lower number of blood transfusions per year (as a consequence of less pronounced anemia), higher hemoglobin level, lower MCV, MCH, and the number of reticulocytes were observed with the increased number of 3.7 kb alpha-thalassemia deletions, with more significant values in the homozygous. The improvement in these hematological indices (namely reduction of anemia and reduction of microcytosis) could be the most important factor that contributes to the less severe outcome of this phenotype and possibly the patient's survival, as suggested by others [23]. In a recent study, also performed in a group of SCD children with Angolan ancestry, it was observed that the group of patients who co-inherited at least one alpha-thalassemia allele had lower levels of reticulocyte count than the other group without alpha-thalassemia. The first group presented a reticulocyte count mean of $8.61 \pm 3.58\%$ while the latter presented $12.85 \pm 4.71\%$ ($p < 0.001$) [25]. Therefore, like these

authors, in this study we confirm that the co-inheritance of alpha-thalassemia ameliorates the hemolytic rate of SCD, subsequently to a reducing mean cell hemoglobin concentration and erythrocyte density, thus decreasing the tendency of deoxy-HbS to polymerize [23, 26]. The possible biological explanation for this association and subsequent clinical beneficial effects to patients is that alpha-thalassemia leads to a reduction in HbS, and consequently a reduction in red blood rigidity and sickling, longer lifespan, lesser microcytosis, lower reticulocyte counts, MCH and MCV, raised hematocrit and blood viscosity [19, 21, 27]. In the present study, this hypothesis is confirmed by the reduction in the number of hospitalizations and stroke events in association with the presence of the alpha-thalassemia deletion.

On the other hand, the association between the presence of alpha-thalassemia and a decreased reticulocyte count, one laboratory biomarker of hemolysis, is positive for the pathophysiology of SCD once free hemoglobin is

a well-known scavenger of nitric oxide, which maintains vasodilation through a cascade of biological events that culminate in the relaxation of smooth muscle cells that line blood vessels. Thus, the co-inheritance of alpha-thalassemia with SCD acts beneficially by lowering reticulocytes count and consequently its wicked adhesion to endothelium, and by decreasing chronic hemolysis level, and consequent free hemoglobin level, preserving the benefit of higher nitric oxide bioavailability [23, 26].

In conclusion, in our study, the prevalence of 3.7 kb alpha-thalassemia deletion in Angolan sickle cell pediatric patients and its association with disease phenotype has been described, being 12.5% of homozygotes and 55.0% of heterozygotes. The number of deletions was associated with less severe phenotypes in these sickle cell Angolans patients. Improved hematological indices, lower blood transfusions, hospitalizations rate and stroke events, contributing to an improvement of the general well-being and probably improving the survival of SCD homozygous for alpha-thalassemia. For the first time in an Angolan population, the results obtained reveal that alpha-thalassemia deletions in SCD influences both hematological and clinical aspects and produces a milder phenotype.

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Author contributions MB, BS, APA, and PF participated in the design of the study; MB, BS, MD, JF, PF, AM, IG contributed to data collection and data analysis; MB, BS, MD, JF, PF, AM, IG, APA, contributed to interpretation of results; MB, BS contributed to manuscript writing; All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

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Data availability That is available under request to the corresponding author.

Compliance with ethical standards

Conflict of interest The authors declare that they do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

Ethics approval This study was approved by the Ethical Committee of the Ministry of Health of Angola (CE. N° 040/2018), the Ethical Committee of ESTeSL (CE-ESTeSL-N° 43-2018).

Consent to participate Informed consent was obtained and signed by the caregivers of children.

Consent to publication All authors transfer the ownership of copyright to the journal Molecular Biology Reports should their work be published in this journal.

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