RESEARCH ARTICLE

Influence of alpha thalassemia on clinical and laboratory parameters among nigerian children with sickle cell anemia

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Background: There is paucity of data on the influence of alpha thalassemia on the clinical and laboratory parameters among Nigerian sickle cell anemia (SCA) patients. This study aimed to determine the prevalence of alpha thalassemia and the influence of alpha thalassemia on laboratory parameters and clinical manifestations in a group of young Nigerian SCA patients.

Methods: This was a cross-sectional retrospective study conducted on 100 patients with SCA and 63 controls. The diagnosis of SCA was confirmed by DNA studies. Alpha thalassemia genotyping was performed by multiplex gap-PCR method. Laboratory parameters including complete blood count, hemoglobin quantitation, serum lactate dehydrogenase (LDH), and bilirubin were determined with standard techniques.

Results: Alpha thalassemia was found in 41 (41.0%) patients compared to 24 (38.1%) controls ($P = 0.744$), and all were due to the 3.7 kb α -globin gene deletions. Alpha thalassemia was associated with more frequent bone pain crisis, higher hemoglobin concentration, red blood cell count, and HbA₂ level among the patients. On the contrary, patients with alpha thalassemia had lower mean corpuscular volume, mean corpuscular hemoglobin, and white blood cell count (WBC) ($P < 0.05$). There were 6 (6.0%) patients with leg ulcers, and none of them had alpha thalassemia, $P = 0.04$.

Conclusion: This study confirms that coexistence of alpha thalassemia with SCA significantly influences both the clinical and laboratory manifestations of young Nigerian SCA patients. The coexistence of this genetic modifier is associated with increased bone pain crisis and protects against sickle leg ulcers among the patients.

KEYWORDS

alpha thalassemia, children, clinical manifestations, laboratory parameters, nigeria, sickle cell anemia

1 | INTRODUCTION

Sickle cell anemia (SCA) is very common among Africans.¹ Globally, about 312,000 neonates are born with SCA annually and 75% of these births occur in Africa.¹ Nigeria has the highest burden of SCA

in the world.² SCA is characterized by heterogeneous clinical phenotypes, and genetic modifiers play roles in the clinical presentation of the disease.^{3,4}

Alpha thalassemia (α -thal) trait secondary to one ($\alpha\alpha$ - $\alpha^{3.7}$) or two ($-\alpha^{3.7}/-\alpha^{3.7}$) α -gene deletion has been found to, partly protect patients with SCA from early hyposplenism,⁵ gallstones,⁶ stroke,⁷ priapism,⁸ glomerulopathy,⁹ and leg ulcers.^{7,10-12} However, other

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authors have reported higher rates of painful crises,¹³ osteonecrosis¹⁴ and retinopathy¹⁴ in SCA patients with α -thal. Although several studies have described the possible effects of α -thal among patients with SCA in the developed world,^{5-7,9-14} there is paucity of information about the influence of this genetic factor in African patients with SCA. Although the few available studies from Africa have established that the 3.7 kb α -globin gene deletion is the common α -thal allele among the African population,¹⁵⁻²⁰ nonetheless, there is lack of consensus on its impacts on the laboratory and clinical manifestations of patients with SCA.¹⁵⁻²⁰

Because of genetic variability in different populations, it is pertinent that more studies are carried out among cohorts from different ethnic backgrounds to fully understand the impact of genetic modifiers on SCA. The aim of this study was to assess the prevalence of α -thal trait and its influence on laboratory parameters and clinical events in a group of young Nigerians with SCA.

2 | PATIENTS AND METHODS

2.1 | Study design and setting

This was a cross-sectional retrospective study conducted on 100 hydroxyurea-naïve SCA children and adolescents who are regular attendees at the pediatric hematology unit of the Ekiti State University Teaching Hospital (EKSUTH), Ado Ekiti, Ekiti State, Southwestern Nigeria.

2.2 | Participants' selection and SCA diagnosis

To qualify for inclusion, the SCA patient must be a regular attendee at the unit and must have been on regular follow-up at the unit for a minimum of 1 year prior to recruitment with up-to-date hospital records. Participants with confirmed or other suspected chronic diseases apart from SCA were excluded. Also excluded were the few SCA patients on regular blood transfusion and hydroxyurea therapy. SCA was initially diagnosed by hemoglobin electrophoresis and later with high-performance liquid chromatography (HPLC) when the facility became available. In addition, SCA was confirmed by DNA studies. Sixty-three apparently healthy children who accompanied their siblings to or attended the outpatients' well-child clinic served as controls.

2.3 | Ethical considerations

Written informed consent of parents/caregivers as well as patients' assents and consents were obtained as applicable. The study was approved by the hospital's Institutional Ethics and Research Committee no: A67/2016/03/003. Because the DNA analysis was performed at the hematology and hemotherapy center, university of Campinas, the study was also approved by the University of Campinas Ethics Committee no: CAAE 54031115.9.0000.5404.

2.4 | Data collection

2.4.1 | Clinical and laboratory data

A pretested chart review form was used to extract relevant information from the patients' hospital records regarding their steady-state laboratory parameters and clinical events. An average of, at least, two steady-state results of laboratory parameters performed between 3- and 6-month intervals were recorded for each participant. The steady-state parameters included the complete blood count performed by Sysmex KX21N Haematology analyzer (Sysmex Corporation, Kobe, Japan); serum lactate dehydrogenase (LDH), bilirubin, and aspartate transaminase (AST) were measured with standard techniques. HbF quantitation was performed using HPLC (Bio-Rad Variant D10, Hercules, CA, USA). Steady state was defined as being free from any acute event(s) for at least 1 month and transfusion free for at least 100 days.²¹

Other information retrieved from patients' charts included their age, sex, and the diagnoses of clinical events in their medical records. The diagnoses of clinical events among all the patients were made from independent reviews by at least two physicians one of whom must be a pediatric hematology specialist. These diagnoses were arrived at using both clinical skills and appropriate investigations. Data documented on the clinical evolution of the patients with respect to the number of bone pain crises requiring hospital visit, admission, and administration of analgesics within the preceding 1 year were retrieved. In addition, the presence or absence of chronic SCA complications including leg ulcers, priapism and overt osteonecrosis or overt stroke were retrieved from the records. The definitions of clinical events were as previously described.²²

2.4.2 | Genetic studies

These were carried out at the Centro de Hematologia e Hemoterapia (Hemocentro), UNICAMP, Campinas, Sao Paulo State, Brazil.

DNA extracted from each participant by Qiagen QIAamp DNA (Blood Mini Kit Cat No 51104, Hilden, Germany) was used to confirm the diagnosis of SCA by polymerase chain reaction (PCR).

2.4.3 | Alpha thalassemia determination

Alpha-thalassemia (α 3.7Kb deletion) was investigated by GAP-PCR according to Dode et al²³ using primers (C2:CCATGCTGGCAGG TTTCTGA and C10: GATGCACCCACTGGACTCT). After electrophoresis in a 1.2% agarose gel, a fragment of 2.1Kb could be observed for normal alleles and 1.9Kb fragment for deleted alleles ($\sim\alpha$ 3.7Kb). Other details are provided in the supplemental materials.

Alpha-thalassemia (α 4.2Kb deletion) was investigated by Multiplex-PCR according to Oron-Karni et al²⁴ using primers (P71: TACCATGTGGTGCCTCCATG, P72:TGTCTGCCACCTCTTCTGAC. And P52: CCTCCATTGTTGGCACATTCC). After electrophoresis in a 1.2% agarose gel, a fragment of 1596 bp was expected for deleted alleles ($\sim\alpha$ 4.2Kb) while 233-bp fragment served as the internal control.

Other details are provided in the supplemental materials. All DNA studies were carried out blinded regarding the clinical and laboratory parameters of the participants.

2.5 | Data analysis

Statistical analysis was performed with the GraphPad Prism Program, version 5 for Windows (San Diego, California, USA). The normal distribution of the quantitative variables was verified by the Kolmogorov-Smirnov, D'Agostino/Pearson Omnibus, and Shapiro-Wilk tests. The frequencies of variables were described. The significance of differences in the quantitative variables between three or more groups was assessed using the Kruskal-Wallis analysis of variance (ANOVA) while the Mann-Whitney test was used to compare quantitative variables between two groups. The chi-square and Fisher's exact tests were used for comparison of categorical variables frequencies as appropriate. Level of significance was set at $P < 0.05$.

3 | RESULTS

The patients consisted of 66 males and 34 females with a median age of 8.5, range 2-21 years. The controls were made up of 22 individuals with HbAS and 41 with HbAA, median age of 8.5, range 2-18 years.

They consisted of 36 males and 27 females. The patients had been on follow-up for a median of 4 years, range 1.5-14 years. There were no differences in the socio-biographic data of patients and controls (Table 1).

3.1 | Allele frequency of alpha thalassemia among patients and controls

None of the participants had the alpha-thal trait due to 4.2Kb α -globin gene deletion. Alpha-thal trait (3.7 kb α -globin gene deletion) was found in 42 (42.0%) patients; 34 with heterozygous deletion ($\alpha\alpha/-\alpha^{3.7}$) and 7 with homozygous deletion ($-\alpha^{3.7}/-\alpha^{3.7}$), while 58 (58.0%) patients had normal genotype ($\alpha\alpha/\alpha\alpha$). There was one case of triplication among the patients. Twenty-five controls comprising 9 (41.0%) HbAS, and 16 (39.0%) HbAA had α -thal trait and these were all heterozygous ($\alpha\alpha/-\alpha^{3.7}$). Also, 38 controls made up of 13 (59.0%) HbAS and 25 (61.0%) HbAA had normal genotype ($\alpha\alpha/\alpha\alpha$). Of the 200 patients' chromosomes analyzed, 150 were $\alpha\alpha$, 49 were $-\alpha$, and one was $\alpha\alpha\alpha$, thus giving gene frequencies of 0.75 ($\alpha\alpha$), 0.25 ($-\alpha$), and 0.005 ($\alpha\alpha\alpha$), respectively. Similarly, the gene frequencies among the controls were HbAS - 0.82 ($\alpha\alpha$), 0.18 ($-\alpha$) and HbAA- 0.80 ($\alpha\alpha$), 0.20 ($-\alpha$) respectively, with no gene triplication ($\alpha\alpha\alpha$) detected and these were not significantly different from the frequencies among the patients (Table 1). Taken together,

TABLE 1 Biodata and frequencies of alpha thalassemia alleles

Parameters	SCA (SS) N = 100 n (%)	AS N = 22 n (%)	AA N = 41 n (%)	Chi-square, degree of freedom χ^2 , df	P value
Age in years					
Median (Range)	8.5 (2-21)	9 (3-17)	8.5 (2-18)	NA	0.888 ^a
Sex					
Male	66 (66.0)	13 (59.0)	23 (56.0)	1.350, 2	0.509 ^b
Female	34 (34.0)	9 (41.0)	18 (44.0)		
Alpha thalassemia present	41 (41.0)	9 (41.0)	16 (39.0)	0.2866, 2	0.866 ^b
Alpha thalassemia absent	58 (58.0)	13 (59.0)	25 (61.0)		
α -globin deletion genotype(s)	SCA (SS) N = 100 n (%)	AS N = 22 n (%)	AA N = 41 n (%)		
$\alpha\alpha/-\alpha$	34 (34.0)	9(41.0)	16 (39.0)	0.327, 2	0.849 ^b
$-\alpha/-\alpha$	7 (7.0)	0 (0)	0 (0)	NA	0.043*
$\alpha\alpha\alpha$	1 (1.0)	0 (0)	0 (0)	NA	
Allele frequency	Total chromosome N = 200 n(frequency)	Total chromosome N = 44 n(frequency)	Total chromosome N = 82 n(frequency)		
$\alpha\alpha$	150 (0.75)	36 (0.82)	66 (0.80)	1.590, 2	0.451 ^b
$-\alpha$	49 (0.25)	8 (0.18)	16 (0.20)	0.822, 2	0.662 ^b
$\alpha\alpha\alpha$	1 (0.005)	0 (0.0)	0 (0.0)	NA	

NB: a = Kruskal-Wallis test (ANOVA), b = Chi-square test, * = Fisher's exact test, NA = Not applicable, SCA = Sickle cell anemia, Significant P value is in bold font.

TABLE 2 Alpha thalassemia Alleles and laboratory parameters

Biomarkers	Alpha thalassemia with Heterozygous deletion N = 34 Median (Range)	Alpha thalassemia with Homozygous deletion N = 7 Median (Range)	SCA with no Alpha Thalassemia Trait N = 58 Median (Range)	P value
Hb (g/dL)	7.6 (6.3-11.2)	8.2 (7.6-10)	7.2 (6.2-11)	0.0199
MCV (fL)	78 (66.9-94)	74 (60.3-101)	84 (56-115)	0.0025
RBC (million cells/ μ L)	2.8 (2.15-3.9)	3.1 (2.7-4.1)	2.7 (1.8-4.8)	0.0264
WBC ($\times 10^3/\mu$ L)	14.7 (6.4-26.2)	7.8 (6.1-13.2)	13.3 (6.1-29.3)	0.0353
Platelet ($\times 10^3/\mu$ L)	358 (108-674)	334 (158-444)	371 (118-832)	0.2156
HbF (%)	8.8 (0.6-28.5)	13.1 (3.7-17.5)	10.7 (2.5-32.3)	0.1364
HbA ₂ (%)	1.7 (0.3-3.8)	2.8 (1.5-4.0)	1.5 (0.2-3.1)	0.0002
MCH (pg)	24.5 (18.6-30.6)	24.4 (15.7-27.1)	26 (15.9-34)	0.0021
Total Bilirubin (mg/dL)	2.1 (0.43-7.7)	1.8 (1.03-8.1)	1.8 (0.8-5.2)	0.729
AST (IU/L)	58 (12-89)	40 (22-89)	43 (7-89)	0.3974
LDH (IU/L)	865 (215-1860)	771 (705-986)	881 (197-1681)	0.6469

NB- One case of Triplication not included, Significant p values are in bold fonts, Test statistics = Kruskal-Wallis ANOVA, Hb-Hemoglobin concentration, RBC-Red blood cells, MCV-Mean corpuscular volume, WBC-White blood cells count, HbF- Fetal hemoglobin, MCH- mean corpuscular hemoglobin, AST-Aspartate transaminase, LDH-Lactate dehydrogenase.

the prevalence of α -thal was not different across the groups (SCA 42 (42%) vs HbAS 9 (41.0) vs HbAA 16 (39.0), $\chi^2 = 0.1068$, $df = 2$, $P = 0.9480$).

3.2 | Comparison of laboratory parameters between patients and controls

There were significant differences between the patients and controls in all laboratory parameters $P < 0.05$ except mean corpuscular hemoglobin (MCH). (Table S1)

3.3 | Effects of alpha thalassemia on hematological indices and other laboratory parameters among patients

Co-inheritance of α -thal was significantly associated with higher hemoglobin concentration (Hb), red blood cell count (RBC), and HbA₂ level. On the contrary, it was associated with lower mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH). Also, the white blood cell count (WBC) was significantly lower in patients with homozygous 3.7 kb α -globin gene deletion compared to the two other groups. No significant differences were observed across α -thal genotypes in the other laboratory parameters (Table 2).

3.4 | Effects of alpha thalassemia on clinical events among patients

Sixty-one (61.0%) patients were admitted for bone pain episodes in the preceding 1 year. Five (5.0%) had overt stroke, 5 (5.0%) had overt

osteonecrosis while 5 (7.5%) males had priapism as chronic complications of their SCA.

The rate of painful crisis was higher in patients with α -thal compared to those without it ($P < 0.0001$). However, the presence of α -thal seems to protect against leg ulcer as none of the six patients with leg ulcer had α -thal 0 (0.0%) vs 6 (10.3%), $P = 0.04$. No significant association was found in relation to other clinical events with respect to the presence or absence of α -thal (Table 3).

4 | DISCUSSION

The two α -globin genes on chromosome 16 are located within a 4-kb duplicated region leading to the possibility of rearrangements including deletions and triplications with many attendant downstream consequences.²⁴ The present study confirmed earlier reports that the 3.7 kb α -globin gene deletion is the common α -thal allele among Africans¹⁵⁻²⁰ as none of the participants had the 4.2Kb α -globin gene deletion.

The prevalence of alpha thalassemia among patients in this study (41%) is comparable to between 37% and 46% earlier described among mixed populations of SCA patients from France¹³ and West Africa.^{15,19,20} This is however higher than the 13%-28% described among patients in the Americas.²⁵⁻²⁸ These observations may be due to regional differences.

The observed hematological profile of patients with α -thal in this study has been previously described.^{10,13,18-20,25,26,28} The increased Hb and red blood cell count are due to the decrease in the intracellular concentration of HbS, and number of dense red blood cells.⁷ These, in turn, lead to increased red blood cell deformability and

TABLE 3 Co-inheritance of sickle cell anemia with Alpha Thalassemia and clinical events

Clinical events	Patients with Alpha Thalassemia (N = 41)	Patients without Alpha Thalassemia (N = 58)	P value
Bone pain crisis per Year	Range (0-6) Median 3	Range (0-6) Median 1	<0.0001[†]
Stroke	2	3	1.000*
Osteonecrosis	3	2	0.646*
Leg ulcer	0	6	0.040*
Priapism n = 66 (Male only)	(N = 34) 1	(N = 32) 4	0.1974*

NB = Significant P values are in bold fonts, [†]Mann-Whitney test, *Fisher's exact test.

decreased rates of both HbS-induced RBC polymerization and hemolysis.^{3,7,14} However, the observed pattern of HbA2 and HbF in the patients could be due to the young age of the patients and the effects of β -globin genes cluster that appear to modulate both HbF and HbA2 in SCA which were not examined in this study.²⁹

Beyond these, the hematologic profile of patients with alpha thalassemia also mimics that of iron deficiency anemia. Hence, the similarities between these two conditions further highlight how alpha thalassemia status of children with SCA could impact their care as it may confuse the clinician and, possibly leads to misdiagnosis and or mismanagement for iron deficiency.³⁰⁻³² This is more important for patients in the developing countries given the high prevalence of both conditions among children in these parts of the world.^{30,32}

Contrary to some earlier reports that alpha thalassemia did not influence the clinical events in SCA,^{26,28} in this study, and in agreement with most published data on this subject,^{4,7,13,14} the presence of α -thal was associated with higher rates of bone pain crisis compared to those without α -thal. This finding is in keeping with that of Renoux et al,¹³ among children with SCA in France and other previous reports.^{7,14} Vaso-occlusive crisis (VOC) manifesting as bone pain crisis is a common complication of SCA that is thought to be associated with increased blood viscosity.^{7,13} Studies have demonstrated that SCA patients with α -thal have increased blood viscosity because of relatively higher levels of Hb and/or hematocrit, among other mechanisms.^{3,7,8,13,14,33} In this study, the SCA patients with α -thal had higher Hb that could lead to increased blood viscosity.

Furthermore, as found in this study, authors from different parts of the world have reported that α -thal protects against leg ulcer in SCA patients.^{10,11,17} However, some have reported that no such association exists.^{15,16} These observations reflect lack of consensus on the roles of α -thal in some phenotypes of SCA. Leg ulcer is a chronic and debilitating complication that is thought to be associated with very severe phenotype of SCA.^{3,12,34-36} Given the high burden of SCA in Africa, there is need for more studies from this part of the world to explain the relationship between α -thal and leg ulcer, and other SCA phenotypes.

The observations that α -thal is associated with lower frequency of stroke in children with SCA^{37,38} was not sustained in this study. On the contrary, one previous study²⁰ found that, in combination with *BCL11A* variants, α -thal was associated with increased risk for stroke in older SCA patients. The lack of association between

α -thal and stroke in this study is also in keeping with report by Filho et al³⁹ Nevertheless, these findings need to be interpreted with caution because of differences in the age of study cohorts and possibility of survival bias. In addition, stroke was determined in this study by overt clinical history and was, in most cases, not confirmed by magnetic resonance imaging or angiography and this could lead to detection bias and exclusion of cases of silent infarcts from our analysis.

The lack of association between α -thal and osteonecrosis in this study contrasts with the report by Milner et al⁴⁰ on some older children enrolled in the cooperative study of sickle cell disease in the USA where they found that, both α -thal and increasing age were significantly associated with osteonecrosis. Possible explanations for our findings included the fact that our study participants were younger compared to that by Milner et al.⁴⁰ The youngest patient in this study was 2 years old, while theirs was 5 years old.

This study was limited by the small sample size and young age of the patients. It is also likely that the apparent higher occurrence of the homozygous alpha thalassemia in the patients relative to the control is not real and may be due to the smaller number of the controls and the small size of this study. Despite these limitations, the study was able to demonstrate that some important SCA phenotypes were influenced by the presence of alpha thalassemia among the patients. Hence, this study represents an important contribution to the scanty literature on the roles of genetic modifiers in the expression of SCA among patients in the developing countries.

In conclusion, this study shows that coexistence of α -thal influences the hematologic parameters of Nigerian children with SCA. It also showed that α -thal was associated with increased rates of bone pain crisis and protects against the occurrence of leg ulcer among the patients.

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CONFLICT OF INTEREST

The authors declare no conflict of interest with respect to this study.

AUTHORS' CONTRIBUTION

OSO, AA, and FFC conceived and designed the study. OSO wrote the article, participated in data collection, analysis, patients recruitment, and follow-up. DMA performed the genetic studies. All authors reviewed the manuscript for important intellectual contents and approved the final version.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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