

Alpha thalassemia among sickle cell anaemia patients in Kampala, Uganda

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Abstract:

Background: Sickle cell anaemia is prevalent in sub Saharan Africa. While α^+ -thalassaemia is known to modulate sickle cell anaemia, its magnitude and significance in Uganda have hitherto not been described.

Objectives: To determine the prevalence of α^+ -thalassaemia among sickle cell anaemia patients in Mulago Hospital and to describe the clinical and laboratory findings in these patients.

Methods: A cross sectional study was carried out on patients with sickle cell anaemia in Kampala. Dried blood spots were used to analyze for the deletional α^+ thalassaemia using multiplex polymerase chain reaction.

Results: Of the 142 patients with sickle cell anaemia, 110 (77.5%) had the $\alpha\alpha$ -thalassaemia deletion. The gene frequency of (- α) was 0.425. Ninety one percent (100/110) of those with α^+ -thalassaemia were heterozygous ($\alpha\alpha/\alpha^-$). Amongst the patients older than 60 months, 15 (83.3%) of those without $\alpha\alpha$ -thalassaemia had significant hepatomegaly of greater than 4 cm compared to 36 (45.6%) of those with α^+ -thalassaemia ($p=0.003$).

Conclusion: The gene frequency of (- α) of 0.425 noted in this study is higher than that reported from many places in Africa. Concurrent alpha thalassemia might be a protective trait against significant hepatomegaly in sickle cell anaemia patients more than 60 months of age at Mulago hospital.

Keywords: Alpha thalassemia, sickle cell anaemia patients, Kampala, Uganda

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Introduction

In the early 1960's many adults with sickle cell anaemia (SCA) as well as those with mild disease were reported in Jamaica¹.

Various factors, both genetic and environmental, are known to influence the clinical course and survival of patients with SCA. These factors do not only include the different haplotypes of sickle cell and infections, but also the interaction of sickle cell with alpha thalassaemia.

About 30% of patients with SCA have concurrent deletional alpha thalassaemia (α^+ -thalassaemia)². The heterozygotes have reduced concentration of HbS, and HbS polymerization, less haemolysis, higher PCV, lower MCV and lower reticulocyte counts³⁻⁵. Alpha thalassaemia tends to ameliorate some but not all of the clinical

features of SCA⁶⁻⁸. It is widespread in Africa and is thought to reflect a survival advantage against severe malaria⁹⁻¹². While there have been reports of α^+ -thalassaemia elsewhere in Africa, there is a dearth of information on its prevalence and interaction with sickle cell anaemia in Uganda¹³⁻¹⁵.

In 1958 Raper described nine cases of thalassaemia major amongst Ugandans of Indian origin¹⁶. The main objective of the current study was to determine the prevalence of α^+ -thalassaemia among SCA patients attending the sickle cell clinic at Mulago national referral hospital Kampala, using multiplex polymerase chain reaction (MPCR), and to describe laboratory and clinical findings in these patients. This paper describes select clinical, and laboratory characteristics of a cross-section of children with SCA.

Methods

The Sickle Cell Clinic at Mulago hospital has over 7000 registered patients with SCA. This cross sectional study was conducted from December 1994 to January 1995.

Sampling and recruitment

Assuming a prevalence of α^+ -thalassaemia of 0.26 based on a Kenyan study⁽¹⁵⁾ and a precision of 6.3% at 95% confidence intervals, every third patient with a

confirmed diagnosis of SCA, was enrolled and data was obtained from 142 children aged up to 19 years.

Basic demographic, anthropometric, and clinical data were collected. Those who were very sick and those who had had a blood transfusion in the previous three months were excluded.

Written informed consent was obtained from the parent or caretaker of each child, and ethical clearance was obtained from the Department of Paediatrics and Child Health, Makerere University and the National Council of Science and Technology.

Laboratory data

Haematological data was obtained for all children using a haematology analyser (Beckman Coulter Inc, AcT Miami FL 33196-2500 USA). Haemoglobin was analysed by electrophoresis on cellulose acetate gels (Helena Laboratories UK limited) at pH8.6 voltage 200v. Migration time was at least 30 minutes, and the strips were labeled using serum as a marker. Dried blood spots for the DNA analysis were kept at a room temperature for at least four hours, stored at 4°C and later transported to the Clinical Biochemistry Laboratory — Evanston Hospital USA, where DNA analysis to type for α -globin genotype was performed using Multiplex Polymerase Chain Reaction (MPCR) techniques¹⁷.

Thick and thin blood smears were analysed for malaria parasites and peripheral blood picture and were performed on all study participants.

Data management and statistical analysis

Epi-info software version 6 was used for data-entry and analysis. The sample was described using frequency distributions, while tables and graphs were used to illustrate variables.

Mean values for continuous variables were reported as ± 2 standards deviations.

The chi squared test was used to determine associations between 'exposure' and the main outcome variables.

Results

A total of 142 patients with SCA were recruited of whom 67 (47.2%) were males.

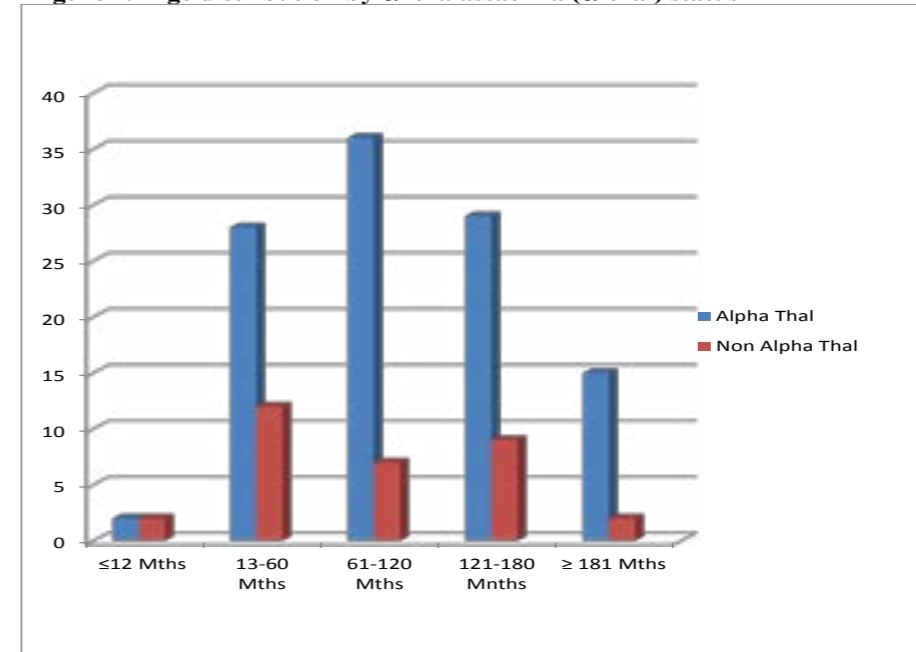
Alpha -thalassaemia status

The gene frequency for (-oc) deletion was 0.425. One hundred and ten participants (77.5%) had α^+ -thalassaemia, while 32 participants (22.5%) had a normal component of alpha genes. Of the 110 participants with α^+ -thalassaemia, 100(90.9%) were heterozygotes (ococ/oc-) while 10 (9.0 %) were homozygotes (oc-/oc-). The majority of the participants with α^+ -thalassaemia were in the age group 61-120 months. Among participants who were more than 120 months the majority 44/55 (80%) were from the α^+ -thalassaemia group.

The Baganda were the predominant ethnic group accounting for 76.1% (108/142), followed by the Basoga with 8.5 % (12/142).

The age range of the participants was 5.9 months to 19 years, with a mean age of 8.7 years and a median of 8.2 years. The age group 61-120 months, had the largest number of study participants (n=43). Fig 1

Figure 1. Age distribution by α^+ thalassaemia (α^+ thal) status



Most participants with α^+ -thal were in the age group 61-120 months.

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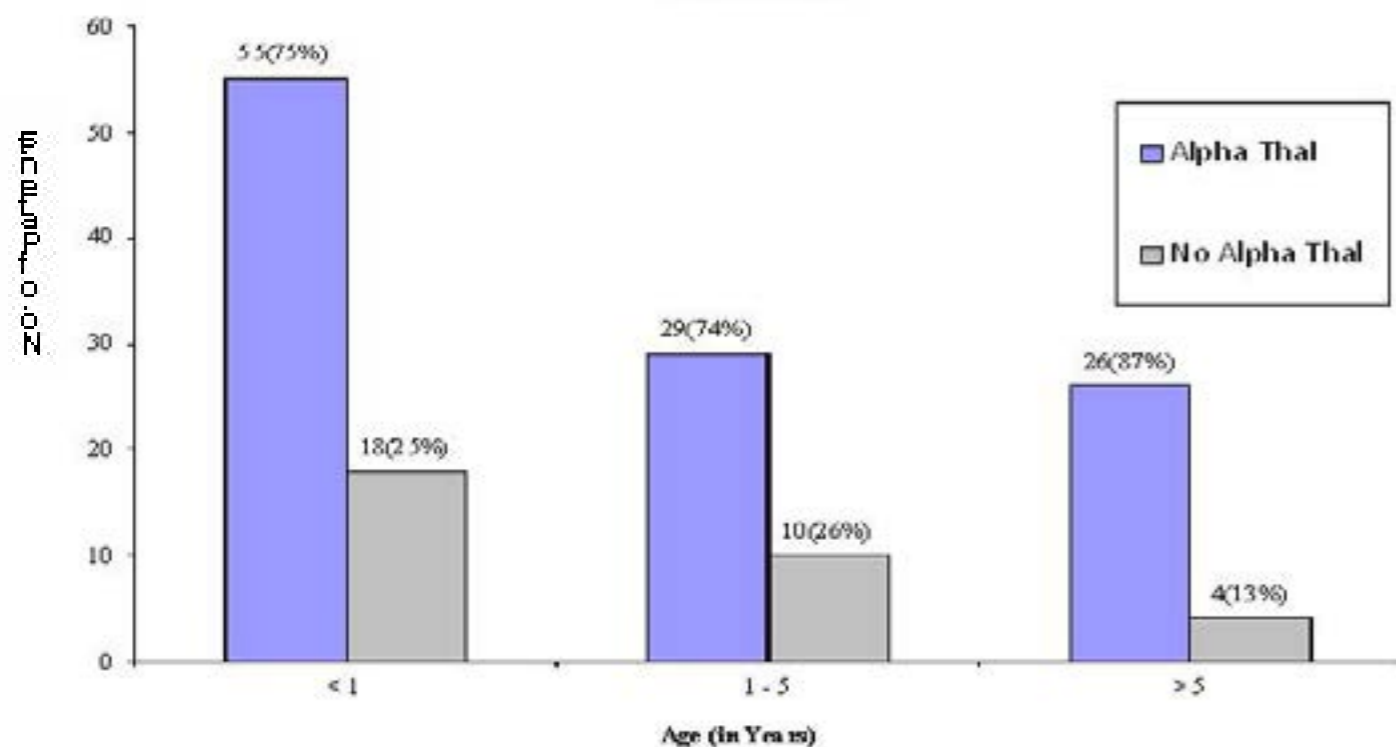
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Age at initial diagnosis

Figure 2 shows the relationship between age at initial diagnosis of SCA and α^+ thalassaemia status. Participants were grouped according to age at which a diagnosis of sickle cell anemia was first made. In about half the cases ((51.4%) (73/142)), the disease presented during the first 12 months of life, with only 21% (30/142) of all the participants first presenting after

the age of 5 years, and by the age of five years 79% had had the diagnosis of SCA made. Alpha thalassaemia did not seem to affect the age at which a diagnosis of SCA was first made ($P=0.39$). Of the 110 participants with α^+ thalassaemia, 26 (23.6%) had the diagnosis of SCA made after 5 years in comparison to only 4 (12.5% in the non- α^+ thalassaemia group.

Fig 2. Distribution of Patients by Age at First Diagnosis and α -thal Status



Clinical history and physical findings of the participants is presented in Table 1 and 2. At the time of the study, 107 (75.3%) of all the participants had had a duration of symptoms of SCA for less than 1 year.

Table 1: History and symptoms of sickle cell patients with or without α^+ thalassaemia (α^+ thal).

| History/Symptom | α^+ thal (n=110) | Non- α^+ thal (n=32) | P value | OR (CI) |
|--|-------------------------|-----------------------------|---------|--------------------|
| Hand and foot syndrome at initial presentation | 69 (65.7%) | 21 (65.6%) | 0.75 | 0.88 (0.36 – 2.16) |
| Severe anaemia at Initial presentation | 14 (13.3%) | 6(18.8%) | 0.39 | 0.63 (0.20 – 2.06) |
| Hand foot syndrome in The last one year | 28 (25.7%) | 10 (31.2%) | 0.52 | 0.75 (0.29 – 1.94) |
| Painful limbs in the last one year | 101 (92.7%) | 26 (81.3%) | 0.087 | 2.59 (0.74 – 8.94) |
| History of hospitalisation | 84 (76.4%) | 26 (81.2%) | 0.56 | 0.75 (0.24 – 2.18) |
| Blood transfusion | 49 (44.5%) | 18 (56.2%) | 0.24 | 0.62 (0.26 – 1.48) |

OR = Odds Ratio CI = Confidence Intervals

Table 2: Findings on physical examination of sickle cell patients and α^+ thalassaemia (α^+ thal) status

| Clinical findings | α^+ thal | Non- α^+ thal | P-Value | OR (CI) |
|---|-----------------|----------------------|--------------|---------------------|
| Height for age | | | | |
| < 2SD | 14 (46.5%) | 5 (36.7%) | 0.77 | 0.79 (0.24 – 2.77) |
| >2SD | 16 (53.5%) | 9(63.3%) | | |
| Weight for age | | | | |
| < 2SD | 12 (40%) | 4 (28.6%) | 0.51 | 0.86 (0.23 – 3.44) |
| >2SD | 18 (60%) | 10(71.4%) | | |
| Weight for height | | | | |
| > 2SD | 4 (13.3%) | 2 (14.3%) | 0.41 | 0.57 (0.08 – 4.70) |
| >2SD | 26(86.7) | 12(85.7) | | |
| Dactylitis in Less than 60 mths of age | | | | |
| Yes | 5 (16.1%) | 1(7.1%) | 1.00 | 1.480.16 – 34.66) |
| No | 26 (83.9) | 13 (92.9%) | | |
| Hepatomegaly in Greater than 60 mths of age | | | | |
| ≤ 4cms | 43 (54.4%) | 3 (16.7%) | 0.003 | 6.20 (1.66 – 27.31) |
| >4cms | 36 (45.6%) | 15 (83.3%) | | |
| Persistent splenomegaly in >more than 60 mths age | | | | |
| Yes | 49(62%) | 1(61.1%) | 0.31 | 1.53 (0.63 – 3.78) |
| No | 30 (38%) | 7 (38.9%) | | |

OR = Odds Ratio. CI= 95% confidence interval. Apart from hepatomegaly > 4cm in α^+ thal subjects more than 60months of age, α^+ thal status did not influence the physical findings.

A history of leg ulceration was available in only 3 participants (ages 12, 16 and 17 years) and only 1 participant (from the non- α^+ thalassaemia) was found to have chronic leg ulceration.

About half of the participants 55.4% (61/110) in the α^+ thalassaemia group and 43.7% (14/32) in the non- α^+ thalassaemia group had no history of blood transfusion.

More than half the patients 55.6% (79/142) had no palpable spleen and the frequency of persistent splenomegaly in patients above the age of 60 months was similar in both α^+ thalassaemia and non- α^+ thalassaemia groups with 62% (49/79) and 61.1% (11/18) in the α^+ thalassaemia and non- α^+ thalassaemia group respectively ($P= 0.94$).

Virtually all participants ((99.3%) (141/142)) had a hepatomegaly (1-15cm). Amongst the participants older than 60 months, 83.3% (15) of those without the α^+ thalassaemia deletion had a hepatomegaly of greater than 4 cm compared to 45.6% (36) of those with the α^+ thalassaemia ($p=0.003$). Table 2

The haematological indices did not differ between the two groups. Table 3

Table 3: Haematological findings amongst sickle cell anaemia and α^+ thalassaemia (α^+ thal) ststus.

| Haematological variables(mean) | α^+ thal | Non- α^+ thal | P-value |
|--------------------------------|-----------------|----------------------|---------|
| (a) Hb gdl ⁻¹ | 7.24 | 7.25 | 0.937 |
| (b) Rbc x 101 ⁻¹ | 2.54 | 2.50 | 0.933 |
| (c) PCV (%) | 23.17 | 22.95 | 0.714 |
| (d) MCV (fl) | 91.44 | 91.41 | 0.616 |
| (e) MCH(pg) | 29.88 | 29.50 | 0.845 |
| (f) MCHC (gdl ⁻¹) | 32.71 | 31.96 | 0.448 |

α^+ thal status did not influence the haematological indices.

Only 20 (14.3%) of 140 participants had malarial parasites detected in their blood. All of them had Plasmodium falciparum 1-10 parasites per 100 thick film fields. Other than Plasmodium falciparum no other malaria parasites were detected and only 3 of these participants were free of any symptoms at the time. The commonest peripheral blood film report documented was hypochromia with poikilocytosis and in all participants, sickle cells were detected. Sixty three participants (44.4%) had marked hypochromia, 64 (45.0%) had moderate hypochromia, while 14 (9.8%) had mild hypochromia. Only one patient was reported to have a normocytic peripheral blood picture.

Discussion

The Baganda were the predominant ethnic group 76.1% (108/142) reflecting the general ethnic composition of patients attending clinics and general wards in Mulago hospital. Ndugwa and Kanyike in their analysis of patient's attendance in the same sickle cell clinic reported a similar percentage of 81%¹⁸. The Baganda have been previously reported to have a high incidence of SCA with a carrier rate of 17%¹⁹. A gene frequency of (- α) of 0.425 recorded in this study is probably one of the highest gene frequencies recorded in sub Saharan Africa and comparable to that of Congo Brazzaville where Mouele, et al recorded a gene frequency of 0.45 among patients with SCA, and

that recorded by Williams et al on the Kenyan coast^{12,20}. Other studies including those of Ojwang et al in Kenya and Falusi et al in Nigeria have recorded frequencies of 0.26 and 0.24 respectively^{15,21}.

The high (- α) gene frequency in the current study compared with others from elsewhere in Africa supports the suggestion by other investigators including Moule that there seems to be a gradient for the (- α) across Africa, the gene frequency being highest in equatorial Africa and lowest in both Northern and Southern Africa¹².

On the other hand this high gene frequency might be attributed to the method used to detect the α^+ thalassaemia deletion. Unlike the MPCPCR technique used in the current study¹⁷, earlier tests were based on imprecise globin synthesis techniques that they could not clearly differentiate between α^+ thalassaemia homozygotes, heterozygotes and normal individuals^{22,23}. The question that inevitably arises is that of the gene frequency in the general population versus that in patients with SCA. Does the HbS gene have an affinity for α^+ -thalassaemia and will the frequency of α^+ -thalassaemia be higher among patients with SCA than in the general population?

Pagnier et al noted that in Senegal, the frequency of α^+ thalassaemia was the same in SCA patients as in non SCA individuals (- α =0.1) whilst in Benin, and Upper

Volta the gene frequency in HbSS individuals of 0.27 was almost twice the gene frequency in the non SCA individuals (0.14)²⁴. In Congo Brazzaville it was noted that the gene frequency for the deletional -oc^{3,7} was 0.40, 0.36, 0.44 and 0.45 in newborns, non-SCA adults, sickle trait and individuals with SCA respectively¹². To investigate whether α^+ thalassaemia status influences age at initial presentation of symptoms of SCA, we used "age at initial diagnosis" as a surrogate marker in the analysis. In spite of this approximation and knowing well that there may have been a variable sequential gap between first symptoms and when a diagnosis of SCA was made, it is noteworthy that by 1 year about half of the participants ((73/142) (51.4 %)), and by 5 years (112/142 (78.9%)) had symptoms of SCA.

Although half of the patients presented with symptoms of SCA during infancy, there were only 4 patients with SCA below one year in this study. After infancy, the overall number of patients rose sharply up to 10 years. These results are comparable to those of an observational study among SCA children in Kenya²⁵. That many children die in infancy before a diagnosis of SCA is made and that the older ones that are seen are a reflection of those who have favorable genetic or environmental factors for survival beyond infancy, might explain this trend. There were fewer participants over the age of 10 years. It was observed that for the patients who were more than 15 years of age, the proportion of individuals with α^+ thalassaemia was greater than that of SCA individuals without α^+ thalassaemia. Whether the presence of α^+ thalassaemia improves survival in SCA patients is not certain.

There have been several studies on the effect of α^+ thalassaemia on survival of patients with SCA but the results are not cosmopolitan. Mears et al has suggested that α^+ thalassaemia is related to prolonged survival while Higgs, Miller and Mouele^{5,12,26,27} did not make the same conclusions. This lack of precision could be partly due to the small number of patients studied as well as nonsystematic and structured collection of clinical data in the different studies. In order to settle this question one would have to study a large group of patients with SCA in the older age group and determine the number of those with and without α^+ thalassaemia but this would have to be done after controlling for other genetic and environmental factors that profoundly affect child survival.

There was no statistically significant correlation between α^+ thalassaemia status and a history of painful limbs in the last one year, and a history or presence of or leg ulceration was virtually missing in this population of SCA patients and so were Vaso-occlusive events that are highly dependent on PCV, such as stroke.

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