Alpha thalassemia among sickle cell anaemia patients in Kampala, Uganda

Irene Lubega, Christopher M Ndugwa, Edison A Mworozi, James K Tumwine

Makerere University College of Health Sciences, Paediatrics and Child Health

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 αα+thalassaemia deletion. The gene frequency of (-α) was 0.425. Ninety one percent (100/110) of those with α+thalassaemia were heterozygous (αα/α-). Amongst the patients older than 60 months, 15 (83.3%) of those without αα+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

DOI: http://dx.doi.org/10.4314/ahs.v15i2.48

Introduction

In the early 1960's many adults with sickle cell anaemia (SCA) as well as those with mild disease were reported in Jamaica[1].

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 counts[3-5]. Alpha thalassaemia tends to ameliorate some but not all of the clinical features of SCA[6-8] . It is widespread in Africa and is thought to reflect a survival advantage against severe malaria[9-12]. 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 Uganda[13-15].

In 1958 Raper described nine cases of thalassaemia major amongst Ugandans of Indian origin[16]. 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.

Corresponding author:
Irene Lubega
Makerere University College
of Health Sciences, Paediatrics and Child Health
Email: ilubega@mujhu.org

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 4ºC 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) techniques[17].

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.

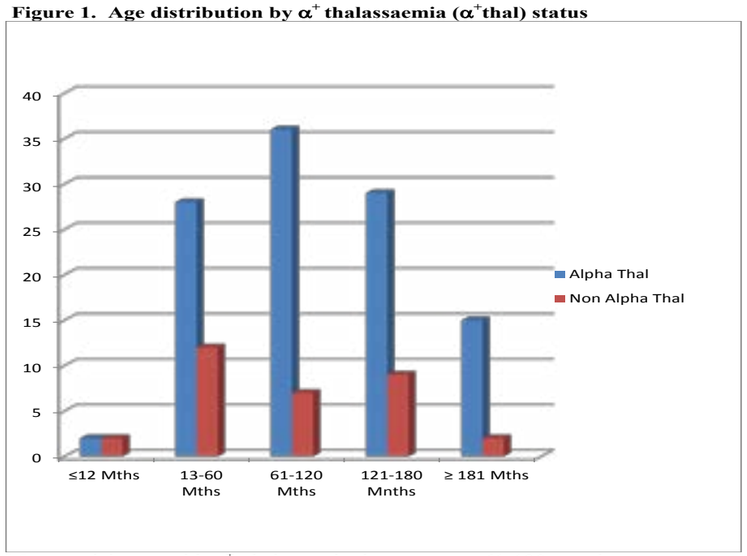
Alpha -thalassaemia status

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.

Age at initial diagnosis

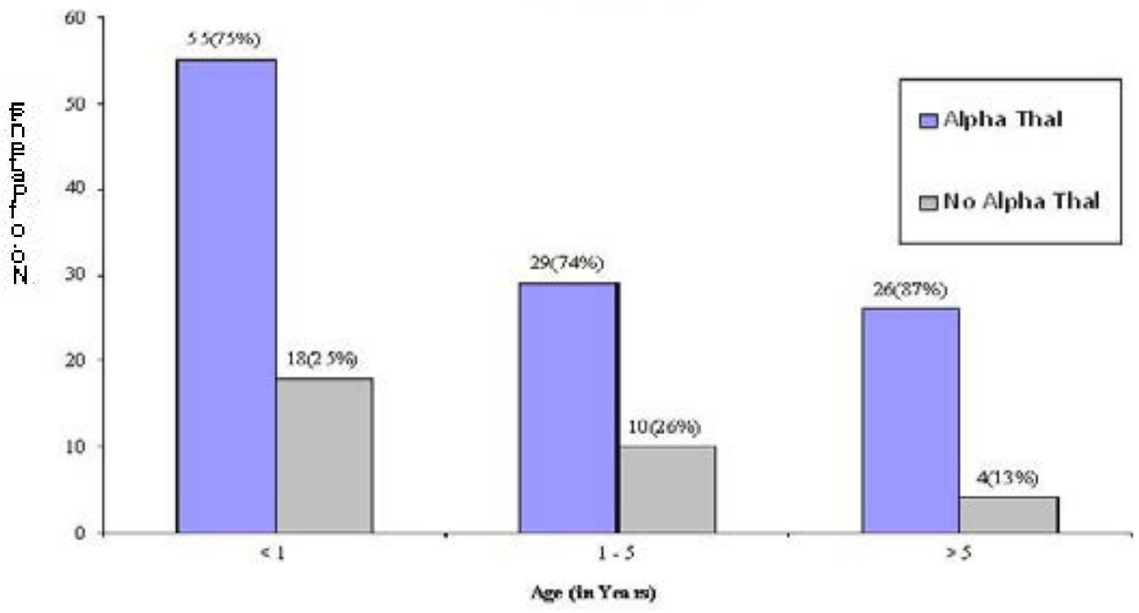
Figure 2 shows the relationship between age at initial diagnosis of SCA and α+thalassaemia status.

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 alpha-thal Status



Clinical history and physical findings of the participants is presented in Table 1and 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				0.57 (0.08 – 4.70)
> 2SD	4 (13.3%)	2 (14.3%)	0.41	
>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		1		
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 falciprarum 1-10 parasites per 100 thick film fields. Other than Plasmodium falciprarum 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 hypocromia, 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 Africa[12].

On the other hand this high gene frequency might be attributed to the method used to detect the α+thalassaemia deletion. Unlike the MPCR technique used in the current study[17], 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 respectively[12]
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 Kenya[25]. 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.

Acknowledgements
We thank the patients and staff of the sickle cell clinic at Mulago hospital, Professor Peter J. Ojwang, University of Nairobi, for their help in this study and the Nuffield foundation and the German Academic Exchange Service (DAAD) scholarship for their financial assistance.

References

1. Serjeant GR, Richards R, Barbor PR, Milner PF. Relatively benign sickle-cell anaemia in 60 patients aged over 30 in the West Indies. *British Medical Journal.* 1968;3(5610):86-91. Epub 1968/07/13.
2. Steinberg MH, Embury SH. Alpha-thalassemia in blacks: genetic and clinical aspects and interactions with the sickle hemoglobin gene. *Blood.* 1986;68(5):985 PubMed -90. Epub 1986/11/01.
3. de Ceulaer K, Higgs DR, Weatherall DJ, Hayes RJ, Serjeant BE, Serjeant GR. alpha-Thalassemia reduces the hemolytic rate in homozygous sickle-cell disease. *The New England Journal of Medicine.* 1983;309(3):189-90. Epub 1983/07/21.
4. Embury SH, Clark MR, Monroy G, Mohandas N. Concurrent sickle cell anemia and alpha-thalassemia. Effect on pathological properties of sickle erythrocytes. *The Journal of Clinical Investigation.* 1984;73(1):116-23. Epub 1984/01/01.
5. Higgs DR, Aldridge BE, Lamb J, Clegg JB, Weatherall DJ, Hayes RJ, et al. The interaction of alpha-thalassemia and homozygous sickle-cell disease. *The New England Journal of Medicine.* 1982;306(24):1441-6. Epub 1982/06/17.
6. Steinberg MH. Predicting clinical severity in sickle cell anaemia. *British Journal of Haematology.* 2005;129(4):465-81. Epub 2005/05/10.
7. van Enk A, Lang A, White JM, Lehmann H. Benign obstetric history in women withsickle-cell anaemia associated with -thalassaemia. *British Medical Journal.* 1972;4(5839):524-6. Epub 1972/12/02.
8. Braden DS, Covitz W, Milner PF. Cardiovascular function during rest and exercise in patients with sickle-cell anemia and coexisting alpha thalassemia-2. *American Journal of Hematology.* 1996;52(2):96-102. Epub 1996/06/01.

9. Enevold A AM, Sanchez JJ , Carneiro I, Roper C,Børsting C,, Lusingu J VL, Lemnge MM,4 Niels Morling N, Riley E and Drakeley CJ. Associations between a+-Thalassemia and Plasmodium falciparum Malarial Infection in Northeastern Tanzania *The Journal of Infectious Diseases*. 2007;196:451–9.
10. Higgs DR, Pressley L, Clegg JB, Weatherall DJ, Serjeant GR. alpha thalassemia in black populations. *The Johns Hopkins Medical Journal*. 1980;146(6):300-10. Epub 1980/06/01.
11. Dozy AM, Kan YW, Embury SH, Mentzer WC, Wang WC, Lubin B, et al. alpha-Globin gene organisation in blacks precludes the severe form of alpha-thalassaemia. *Nature*. 1979;280(5723):605-7. Epub 1979/08/16.
12. Mouele R, Pambou O, Feingold J, Galacteros F. alpha-thalassemia in Bantu population from Congo-Brazzaville: its interaction with sickle cell anemia. Human heredity. 2000;50(2):118-25. Epub 2000/05/09.
13. Henni T, Bachir D, Tabone P, Jurdic P, Godet J, Colonna P. Hemoglobin Bart's in Northern Algeria. *Acta Haematologica*. 1981;65(4):240 PubMed -6. Epub 1981/01/01.
14. Muklwala EC, Banda J, Siziya S, Atenyi J, Fleming AF, Higgs DR. Alpha thalassaemia in Zambian newborn. Clinical and *Laboratory Haematology*. 1989;11(1):1-6. Epub 1989/01/01.
15. Ojwang PJ, Ogada T, Beris P, Hattori Y, Lanclos KD, Kutlar A, et al. Haplotypes and alpha globin gene analyses in sickle cell anaemia patients from Kenya. *British Journal of Haematology*. 1987;65(2):211-5. Epub 1987/02/01.
16. Raper AB. Thalassaemia in families of Indian origin. *East African Medical Journal*. 1958;35(4):161-70. Epub 1958/04/01.
17. Bowie LJ, Reddy PL, Nagabhushan M, Sevigny P. Detection of alpha-thalassemias by multiplex polymerase chain reaction. *Clinical Chemistry*. 1994;40(12):2260 PubMed -6. Epub 1994/12/01.
18. Ndugwa CM, Kanyike FB. Analysis of patients attending sickle cell anaemia clinic, New Mulago Hospital, 1970-1971. *East African Medical Journal*. 1973;50(4):189-98.Epub 1973/04/01.
19. Lehmann H, Raper AB. Distribution of the sickle-cell trait in Uganda, and its ethnological significance. *Nature*. 1949;164(4168):494 PubMed. Epub 1949/09/17.
20. Williams TN WS, Uyoga S, et al. . Both heterozygous and homozygous alpha+ thalassemias protect against severe and fatal Plasmodium falciparum malaria on the coast of Kenya. . *Blood*. 2005;106 368–71.
21. Falusi AG, Esan GJ, Ayyub H, Higgs DR. Alpha-thalassaemia in Nigeria: its interaction with sickle-cell disease. *European Journal of Haematology*. 1987;38(4):370-5. Epub 1987/04/01.
22. Honig G.R Koshy MM, R.G Vida, L.N. Sickle cell syndromes. II. The sickle cell anemia-alpha-thalassemia syndrome. *The Journal of Pediatrics*. 1978:556-61.
23. Felice AE, Webber B, Miller A, Mayson SM, Harris HF, Henson JB, et al. The association of sickle cell anemia with heterozygous and homozygous alpha-thalassemia-2: in vitro HB chain synthesis. *American Journal of Hematology*. 1979;6(2):91-106. Epub 1979/01/01.
24. Pagnier J, Dunda-Belkhodja O, Zohoun I, Teyssier J, Baya H, Jaeger G, et al. α-Thalassemia among sickle cell anemia patients in various African populations. 1984.
25. Manish Sadarangani JM, Albert N. Komba TA-A, Charles R. Newton KMa, Williams TN. An observational study of children with sickle cell disease in Kilifi, Kenya. *British Journal of Haematology*.Volume 146(Issue 6): 675–82.
26. Mears JG, Lachman HM, Labie D, Nagel RL. Alpha-thalassemia is related to prolonged survival in sickle cell anemia. *Blood*. 1983;62(2):286-90
27. Miller ST, Sleeper LA, Pegelow CH, Enos LE, Wang WC, Weiner SJ, et al. Prediction of adverse outcomes in children with sickle cell disease. T*he New England Journal of Medicine*. 2000;342(2):83-9. Epub 2000/01/13.
28. Miller ST, Rieder RF, Rao SP, Brown AK. Cerebrovascular accidents in children with sickle-cell disease and alpha-thalassemia. *The Journal of Pediatrics*. 1988;113(5):847-9. Epub 1988/11/01.
29. Adekile AD,Tuli M, Haider MZ, Al-Zaabi K, Mohannadi S, Owunwanne A. Influence of alpha-thalassemia trait on spleen function in sickle cell anemia patients with high HbF. *American Journal of Hematology*. 1996;53(1):1-5. Epub 1996/09/01.
30. Wali YA, Al-Lamki Z, Hussein SS, Bererhi H, Kumar D, Wasifuddin S, et al. Splenic function in Omani children with sickle cell disease: correlation with severity index, hemoglobin phenotype, iron status, and alpha-thalassemia trait. *Pediatric Hematology and Oncology*. 2002;19(7):491-500. Epub 2002/09/10.
31. Hsu LL, Miller ST, Wright E, Kutlar A, McKie V, Wang W, et al. Alpha Thalassemia is associated with decreased risk of abnormal transcranial Doppler ultrasonography in children with sickle cell anemia. *Journal of Pediatric Hematology/Oncology*. 2003;25(8):622-8. Epub 2003/08/07.
32. Belisario AR, Rodrigues CV, Martins ML, Silva CM, Viana MB. Coinheritance of alpha-thalassemia decreases the risk of cerebrovascular disease in a cohort of children with sickle cell anemia. *Hemoglobin*. 2010;34(6):516 PubMed -29. Epub 2010/11/17.
33. Koshy M, Entsuah R, Koranda A, Kraus AP, Johnson R, Bellvue R, et al. Leg ulcers in patients with sickle cell disease. *Blood*. 1989;74(4):1403 PubMed -8. Epub 1989/09/01.
34. Platt OS, Thorington BD, Brambilla DJ, Milner PF, Rosse WF, Vichinsky E, et al. Pain in sickle cell disease. Rates and risk factors. *The New England Journal of Medicine*. 1991;325(1):11-6. Epub 1991/07/04.
35. Billett HH, Nagel RL, Fabry ME. Paradoxical increase of painful crises in sickle cell patients with alpha-thalassemia. *Blood*. 1995;86(11):4382 PubMed . Epub 1995/12/01.
36. Steinberg MH, Rosenstock W, Coleman MB, Adams JG, Platica O, Cedeno M, et al. Effects of thalassemia and microcytosis on the hematologic and vasoocclusive severity of sickle cell anemia. *Blood*. 1984;63(6):1353 PubMed -60. Epub 1984/06/01.
37. Mukherjee MB, Surve R, Tamankar A, Gangakhedkar RR, Ghosh K, Lu CY, et al. The influence of alpha-thalassaemia on the haematological & clinical expression of sickle cell disease in western India. *The Indian Journal of Medical Research*. 1998;107:178-81. Epub 1998/05/30.
38. Darbari DS, Onyekwere O, Nouraie M, Minniti CP, Luchtman-Jones L, Rana S, et al. Markers of severe vaso-occlusive painful episode frequency in children and adolescents with sickle cell anemia. *The Journal of Pediatrics*. 2012;160(2):286-90. Epub 2011/09/06.
39. Kulozik AE, Kar BC, Serjeant GR, Serjeant BE, Weatherall DJ. The molecular basis of alpha thalassemia in India. Its interaction with the sickle cell gene. *Blood*. 1988;71(2):467-72. Epub 1988/02/01.
40. Olatunji PO, Falusi AG. Persistent hepatomegaly: an index of severity in sickle cell anaemia. *East African Medical Journal*. 1994;71(11):742-4. Epub 1994/11/01.
41. Kaine WN. Sickle cell anaemia in children in Eastern Nigeria. A detailed analysis of 210 cases. *East African Medical Journal*. 1982;59(11):742-9. Epub 1982/11/01.
42. Kahirimbanyi JE. Haematological finings in steady state sickle cell anaemia patients [Thesis]: Makerere University 1976.
43. Coutinho GA. Iron status in Ugandan sicklers [Msc Physiology]: Makerere University; 1987.
44. Adekile AD, Huisman TH. Level of fetal hemoglobin in children with sickle cell anemia: influence of gender, haplotype and alpha-thalassemia-2 trait. *Acta Haematologica*. 1993;90(1):34 PubMed -8. Epub 1993/01/01.
45.Ndugwa C, Higgs D, Fisher C, Hambleton I, Mason K, Serjeant BE, et al. Homozygous sickle cell disease in Uganda and Jamaica a comparison of Bantu and Benin haplotypes. *The West Indian Medical Journal*. 2012;61(7):684-91. Epub 2013/04/30.