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Clinical epidemiology of individuals with Sickle cell anemia using Hydroxyurea at Muhimbili National Hospital, Dar Es Salaam, Tanzania

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Abstract

Background
The pathophysiology of sickle cell disease (SCD) is complex and involves nitric oxide depletion, increased inflammation/adhesion molecules and vaso-occlusion in addition to the chronic hemolytic anemia. This pathophysiology results in systemic clinical complications including recurrent episodes of severe pain, stroke, acute chest syndrome (ACS) and an increased susceptibility to infection. SCD severity varies among individuals and fetal hemoglobin (HbF) is known as a major modulator of the disease. To date, hydroxyurea (HU) is a known intervention that acts by increasing HbF in individuals with SCD. The increase in HbF reduces the risk of 'sickling' events and improves clinical outcomes. This is the first study on the use of HU in individuals with SCA in Tanzania.

Methods
A case-control study to determine the proportion, indications, clinical and laboratory outcomes of SCD patients with HU use was conducted at Muhimbili National Hospital in Dar Es Salaam, Tanzania.

Results
Forty-two patients with Sickle cell anemia (SCA) on HU treatment and 32 patients with SCA not on HU treatment were enrolled. The proportion of HU use by individuals with SCA at Muhimbili National Hospital was 10 per 1000. The mean HbF % was 9.8 ± 2.4 vs 6.2 ±1.4 for controls (P <0.001). Thirty (71.4%) were enrolled for HU treatment due to central nervous system (CNS) events, frequent painful crises 11(26.2%) and recurrent anemia 1(2.4%). Thirty-two SCA patients (76.2%) reported improvements after being on HU for at least six months. Of these, 91% reported no history of severe pain that required hospitalizations since they started HU. Twenty patients (66.7%) out of those with CNS events reported not to have experienced convulsions after HU initiation.

Conclusions
HbF was higher in patients who were on HU and had positive correlation with clinical outcomes. Further clinical trials are required to evaluate more effects of HU use among SCA individuals in Tanzania.

Keywords: Sickle cell anemia, HU, Fetal hemoglobin, Tanzania.

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Key points

- This is the first study of Hydroxyurea use in SCA patients in Tanzania.
- The level of HbF was higher in SCA individuals who were using HU compared to SCA controls in Tanzania. But was lower compared to studies done in USA.
- Hemoglobin level was high in SCA patients who were on HU treatment compared to those who were not on HU treatment.
- Most of our study individuals were black from Central Africa which is known to have Bantu haplotype and have poor response to HU.
- The high level of Fetal Hemoglobin had positive correlation with clinical outcomes.

Introduction

Sickle cell disease (SCD) is a hemoglobinopathy characterized by red blood cells (RBC) which are rigid, abnormal and sickled in shape[1]. Abnormal RBCs result from a point mutation at the sixth codon of the β-globin gene located on chromosome 11. It constitutes the genotypes in which at least 50% of the total hemoglobin being hemoglobin S (HbS) [2]. The most severe and common form is sickle cell anemia (SCA) which contains homozygotic HbSS[2]. In the gene for HbS, adenine replaces thymine leading into substitution of glutamic acid by valine in the 6th position of the β-globin chain[3, 4].

Sickle cell anemia affects millions of individuals worldwide especially those of African origin, India, Mediterranean countries and Spanish speaking countries in south and Central America[5]. In Africa it is estimated that 230,000 children are born with SCA every year[6]. SCA has a high prevalence in Sub-Saharan Africa, with up to 11,000 births of SCA patients per year occurring in Tanzania[8]. Tanzania therefore ranks fourth in the world with highest birth prevalence of SCA.

The clinical manifestations of SCA typically become evident in the first 2 to 4 months of life when fetal hemoglobin (HbF) is replaced with HbS[7, 9]. Hemolysis develops gradually over this period and results in hemolytic anemia, other manifestations are more common after 5 to 6 months of life[1,7].The pathophysiology of SCA is complex and involves nitric oxide depletion, increased inflammation/adhesion molecules and vaso-occlusion in addition to the chronic hemolytic anemia. These in turn result in systemic clinical complications including recurrent episodes of severe pain, stroke, acute chest syndrome (ACS), avascular necrosis of the heads of femurs and humerus and an increased susceptibility to infection.[2, 7]

Treatment of patients with SCA is largely supportive with blood transfusion and HU. HU represents the only widely used drug which modifies disease pathogenesis [25]. HU has been reported to be effective in reducing morbidity and improving survival in some SCA patients. This is due to its effectiveness in increasing HbF which in turn improves hematological values and reduce clinical severity [13, 14]. The clinical outcomes include reduction in frequency of painful crises and hospital admissions [10]. HU has also been found to be effective in the prevention of brain injury due to cerebrovascular diseases [11] in a study done at Duke University Medical Center, Durham, North Carolina USA by Ware et al. However, the effectiveness of HU in some adults has not been shown, raising questions of compliance issues or the possibility of bone marrow exhaustion[12]. Patients from East Africa (like those involved in this study) are known to have most of the Central African Republic (CAR or bantu) haplotype which is known to have lower HbF levels and poor response to HU compared to other haplotypes.[19, 20]

There have been reports of HU adverse effects such as leucopenia, neutropenia, thrombocytopenia, leg ulcers and skin manifestations such as dryness.[15, 16]. HU was approved by the United States Food and Drug Administration (FDA) for treatment of SCA in adults in 1998 and has also become widely used for management of children with SCA in high-income countries [7].

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A research programme was established within the Muhimbili Sickle Cohort (MSC) in Tanzania to evaluate the genetic and environmental determinants of disease severity in SCA. The cohort started in 2004, and has up to 2015 identified over 4,000 SCA individuals. Some of patients from the cohort were started on HU but their clinical epidemiology was not certain. In this report we present an initial description of the clinical epidemiology of SCA individuals who ware on HU. The objective of this study was to determine the proportion, indications and outcome of patients with SCA on HU therapy at Muhimbili National Hospital (MNH).

Methods

Study area, design and population

The study was conducted at the Sickle cell clinic at MNH in Dar es salaam, Tanzania under the Muhimbili Sickle Cell Cohort (MSC) programme.

The MSC consists of prospective surveillance of SCA individuals in Dar es Salaam, Tanzania. It started in 2004, and has identified over 4,000 SCA individuals. It ran sickle cell clinic three times a week, treating an average of 300 to 350 patients monthly.

This was a –case-control study to determine the clinical epidemiology of SCA patients who were on HU. The study was conducted from July 2015 to May 2017. Patients were started on HU due to either of the following indications; frequent painful crises, history of stroke or high risk of stroke from high cerebrovascular velocity, persistent velocity, persistent low hemoglobin and recurrent blood transfusions. Patients were enrolled from the pediatric and adult clinics. Social demographic data, history of painful episodes, recurrence of stroke, convulsions and frequency of blood transfusion were collected. Laboratory parameters measured were HbF, hemoglobin (Hb), red blood mean cell hemoglobin (MCH), red blood

mean cell volume (MCV), reticulocyte counts, decrease in white blood cell count (WBC) and platelets.

The inclusion criteria were patients with SCA on HU for at least 3 months. Comparison group included age and sex-matched individuals with SCA who were seen during this period but were not on HU. Blood samples were collected in EDTA tubes and full blood picture was determined by automated hematology analyzer (Sysmex XT 2000i Kobe, Japan). HbF quantification was determined by high performance liquid chromatography (HPLC) (Bio-Rad Variant I, USA) using the Beta thalassaemia short programme and reported as a percentage of total hemoglobin.
This study was approved by the Muhimbili University of Health and Allied Sciences Research and Ethics committee and permission to conduct this study was given by MNH authority.

Statistical analysis

Data were entered using double entry system. Analysis was done using SPSS (Statistical Package for Social Science) software version 17. The mean levels and P-values of HbF, Hb, MCV, MCH, WBC, and Platelets were determined by the unpaired t-test. P- Value < 0.05 was considered to be statistically significant.

Results

Ninety-seven patients with SCA were recruited into the study. Out of them, 65 reported to have ever used HU and 32 were controls. Out of the 65 patients, 23 were excluded from the final analysis because they were not using HU at the time of the study (*Reasons given provided in* ***Table 2***). The proportion of HU use for SCA at MNH was calculated as the proportion of 65 patients who has ever used HU divide by 4000 SCA patients in the MSC cohort and was found to be 16 per 1000.

Indications for initiating HU are shown in **Table 1** below. The starting dose of HU was found to be 15mg/kg/day and the maximum dose given was 20mg/kg/ per per day orally.

Table: 1. Frequencies of Indications for HU use

Indications	Frequency	Percentage
Stroke	22	52.4
Peripheral neuropathy	1	2.4
Convulsions	6	14.3
Loss of speech	1	2.4
Anemia	1	2.4
Pain	11	26.2
Total	42	100

Data above are presented as frequencies for indications of HU use at Muhimbili National Hospital, Tanzania

Table 2: Reasons for Discontinuing using Hydroxyurea

Number (%)	Reasons
1(4.3)	Severe Leucopenia
1(4.3)	Non response despite a maximum dose of HU (30mg/kg/day)
8(34.7)	HU unavailable in their respective regions
6(26.1)	Unable to afford the medication
2(8.7)	The convulsions had stopped and parents thought their child are cured, and thus no need to continue medication
5(21.9)	Counselling on the duration of medication usage not clearly understood
Total 23(100)	

Clinical outcome

Out of 11 patients who were started on HU due to frequent painful episodes, 10(90.9%) reported no further severe painful crisis requiring admission compared to 12(37.5%) in the control group. The difference was statistically significant, $p<0.001$. Twenty patients (66.7%) out of those with CNS events reported that they did not experience convulsions and have marked motor improvement after HU initiation. One patient who was initiated on HU due to peripheral neuropathy reported no

further numbness or tingling sensation. The patient who was enrolled due to frequent blood transfusions (more than three times a year) did not require further blood transfusions after being transfused since she started using HU (11 months without transfusion at the time of the study).

Table 3: Demographic and laboratory characteristics of individuals with sickle cell anemia (HbSS) who were on HU and controls.

Variable	SCA on HU (n= 42)	SCA not on HU (n = 32)	P value
Male	24	18	
Age (Years)	13.6 ± 10	16 ± 11	<0.001
HbF (%)	9.8 (±2.4)	6.2 (±1.4)	<0.001
Hb (g/dl)	7.5 (±0.4)	6.8 (±0.6)	0.024
MCV (fl)	83.7(±3.5)	80.2(±8.2)	<0.001
MCH (pg)	29.3(±1.3)	27.83 (±3.1)	<0.001
Retics (%)	9.4 (±1.8)	7.2 (±0.9)	0.067
WBC (10^3/µl)	11.3 (±1.3)	11.5 (±3.2)	0.724
PLT (10^3/µl)	409.5 (±64.6)	412x (±40)	0.767

Data above is presented as Tanzania SCA on HU and SCA not on HU population. HbF: fetal hemoglobin, Hb: total hemoglobin, MCV: mean cell volume, MCH: mean cell hemoglobin, Retics: Reticulocytes, WBC: white blood cells, PLT: platelets. SD: Standard deviation.

Discussion

The MSC has enrolled 4,000 patients with SCA out of which 65 were identified to have ever used HU but only 42 were on HU at the time of the study. This makes the proportion of HU use at MNH be 16 per 1000.Twenty-three patients were excluded because they were not using HU at the time of the study. The main indication for

initiating HU was CNS events followed by frequent painful events. Only one patient was enrolled due to severe anemia.

In this cohort 90% of patients with SCA who were started on HU due to frequent painful crises requiring admission, reported no further admissions compared to 38% in the controls. Similar findings were reported in the Multicenter study of HU in SCA which showed lower rates of painful crises in patients on HU compared to placebo (median 2.5 vs 4.5 crises per year, p value <0.001) [10]. HU has been reported to be effective in reducing morbidity and improving survival in SCA patients. The clinical outcomes include reduction in frequency of painful crises and hospital admissions[10]. HU has also been found to be effective in the prevention of brain injury due to cerebrovascular diseases [11].

In this study two thirds of patients who had seizures and stroke events reported no history of further repeated convulsion/seizures or recurrence of stroke since they started using HU. A previous study by *Ware RE et al* found that modified HU therapy can be used as alternative to blood transfusion to prevents stroke recurrence[11,13]. Results of our study cannot be concluded to confirm this effectiveness of HU on CNS events, since these patients were also on the top-up transfusion therapy and physiotherapy.

The mean HbF in patients on HU was significantly higher than in the controls (9.8±2.4 vs 6.2±1.4, p value <0.001). Similar findings were observed in other studies [Ref]. even though in these cohorts the HbF was higher than the MNH cohort (20.6±8). Previous studies of HU in patients with SCA by Ware RE et al and Steinberg MH, et al have documented its effectiveness in increasing HbF parameters, improving hematologic values, and reducing clinical severity [13, 14]
In this study we found the mean value of HbF to be 9.8 %(±2.4%) for SCA patients who were on HU which appear to be high compared to that controls [6.2 (±1.4)] *P<0.001*. Though HbF was higher compared to that of controls it is not comparable

with results of the study done by *Ware et al* which showed Maximum percentage of HbF was 20.6 % (± 8.0%) [13] in individuals who were on HU. The HbF (6.2%) of controls seems to confirm the results of the study done in Tanzania which showed the mean HbF of 6.3% for SCA individuals at Muhimbili Cohort [18].

Findings from the BABY HUG study showed a larger increase in HbF compared to our study[Ref]. This can be explained by the fact that: i) Most of our study patients are from East Africa where the CAR or bantu haplotype is common. Populations with the CAR haplotype are known to have lower HbF levels and poor response to HU compared to other haplotypes.[19, 20] ii) This was an observational study which raises the question of compliance to our patients.

The mean Hb was 7.5g/dl (±0.4) for SCA who were on HU as compared to that of controls 6.8g/dl (±0.6) but the difference was not statistically significant *(P =0.024*). HU has been reported to influence the haemoglobin level simply because intraerythrocytic HbF improves RBC survivals[21].

The MCV and MCH were both higher among cases compared to controls and the differences were statistically significant (P <0.001). These results were expected as high HbF accelerates differentiation of erythropoiesis leading to release of young RBCs and hence larger MCV.[22]

HU is known to reduce WBC, platelets and Reticulocyte counts [15, 16,] but in our study these adverse effects did not appear. There were no significant differences in mean value of WBC, Platelets and reticulocyte counts between cases and controls. This study was limited by sample size; it included only 42 SCA individuals who were on HU and 32 controls. Again, it was an observational study which raises the question of compliance and biasness. We therefore recommend clinical trials to explore more effects of hydroxyurea on SCD patients from Tanzania.

Conclusion

A very small proportion of SCA individuals are using HU at MNH. Fetal hemoglobin was found to be higher in patients who were on HU as compared to controls. There was a positive correlation with clinical outcome of which almost all patients with frequent painful crises reported the reduction of pain severity. Also, the risk of secondary stroke recurrence was reduced markedly. Most of our study individuals were black from Central Africa which is known to have Bantu haplotype and have poor response to HU. Therefore, further clinical trials are required to evaluate more effects of HU in SCA individuals in Tanzania.

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Author Contribution

Elisha Osati designed the research, collected data, analyzed the results and wrote the manuscript. Edward Kija, Furahini Tluway, Magdalena Lyimo, Siana Nkya Mtatiro, and Julie Makani reviewed the results, contributed to analysis and commented on draft manuscripts. Harvest Mariki, Josephine Mgaya, Florence Urio and Musa Makongoro contributed to laboratory data collection. Bruno Mmbando and Raphael Sangeda managed the data, analyzed the results and commented on draft manuscripts. Evarist Msaki managed data and commented on draft manuscripts. Stella Rwezaula, Deogratias Soka and Christina Kindole collected data and commented on draft manuscripts. Julie Makani designed the research, contributed to analysis and commented on draft manuscripts. The authors have read and approved the final manuscript.

Conflict-of –interest disclosure

The authors declare no financial or any other interest.

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