NEONATAL SCREENING FOR HAEMOGLOBINOPATHIES AT MUHIMBILI NATIONAL HOSPITAL, DAR ES SALAAM, TANZANIA

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NEONATAL SCREENING FOR HAEMOGLOBINOPATHIES AT MUHIMBILI NATIONAL HOSPITAL, DAR ES SALAAM

BY

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by Muhimbili University of Health and Allied Sciences a dissertation entitled: **"*Neonatal Screening for haemoglobinopathies at Muhimbili National Hospital, Dar es Salaam*"** in partial fulfilment of the requirements for the degree of Master of Medicine (Haematology and Blood Transfusion) of the Muhimbili University of Health and Allied Sciences.

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ABSTRACT

Background: Neonatal screening for abnormal haemoglobins has been reported as an important intervention in early detection and prompt management for those who are significantly affected and those who are carriers. If associated with clinical care programmes, they have proven to be an important measure at decreasing mortality and morbidity in many regions, as well as providing genetic counselling to parents who are contemplating having children.

Objectives: To determine the frequency of occurrence and types of abnormal haemoglobins in neonates attended at Muhimbili National Hospital, Dar es Salaam.

Materials and Methods: A hospital based cross sectional design was used to recruit neonates attended at MNH from September to November 2009. Eligible neonates were consecutively enrolled after their parents provided informed consent for participation. A structured interview proforma was used to collect information while blood specimens were drawn into EDTA with microgard closure tubes from a heel prick under aseptic techniques. Blood specimens were analysed by HPLC to determine abnormal haemoglobins in whole blood; Alkaline Hb electrophoresis as a second line (confirmatory) test for positive results from HPLC; and ABX PENTRA 60 for haematological parameters (CBC) analysis were used. SPSS version 16.0 was used for data analysis.

Results: A total of 2,053 neonatal samples (mean age = 1.2 $\pm$ 0.5 days and 55.4% males) were analyzed. The frequency of occurrence of abnormal haemoglobins was found to be 18.2% (n=374). The types of abnormal haemoglobins included 12.6% (n=258) with sickle cell trait (Hb FAS); 5.3% (n=109) with possibly α-thalassaemia (Hb Barts); 0.9% (n=19) as sickle cell carrier or Hb S Beta^{+}-thalassaemia (Hb FSA); 0.54% (n=11) had SCA or Hb S Beta^{0}-thalassaemia (Hb FS); and one had Hb FA-D variant.

The frequency of occurrence of abnormal haemoglobins were highest among 35.6% (n=133) and 10.2% (n=38) of participants whose parents origins were reported from Costal Region and Lake Zone respectively, while 6.7% (n=25) of participants from the Northern Region of Tanzania had the lowest frequency of occurrence ($X^2 = 37.7$, $p < 0.01$).

Presence of Hb Barts increased by nearly six times the likelihood of MCV being less than 100 fl in 78.2% (n=68) of participants compared to 14%(n=206) without Barts (X^2 =2.3, $p<0.01$); and increased by 1.3 the likelihood of low birth weight in 33.9% (n=37) compared to 25.4% (n=493) of those without Barts (X^2=7.1 $p<0.05$).

Conclusion: The frequency of occurrence of abnormal haemoglobins amongst neonates participated in the study is high and therefore it may be considered as major public health problem. There are other Hb variants such as Hb Bart, FSA and Hb-D variant which exist amongst study population in addition to FS and FAS which were previously known.

Recommendations: Neonatal screening for abnormal haemoglobins should widely be implemented to allow for early identification and opportunities for comprehensive care. Molecular tests to confirm abnormal haemoglobins detected during screening programme should be emphasized. Larger neonatal cohort studies to generalize the findings of this screening pilot are also recommended.

LIST OF TABLES / ILLUSTRATIONS

Table 1	Types of haemoglobins	23
Table 2	Hb Electrophoresis results following HPLC analysis	24
Table 3	Characteristics of the study population	27
Table 4	The association between SCD and demographic characteristics	28
Table 5	Factors associated with presence of Hb Barts	30
Table 6	Profile of RBC value by sex and age	31
Table 7	Profile of WBC and platelet counts by sex and age	32
Figure 1	Flow chart procedure during study period	22
Figure 2	Map of Tanzania showing the frequency of distribution of abnormal haemoglobins	26

LIST OF ABBREVIATIONS

EDTA	Ethylene Diamine Tetra-Acetic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
HPFH	Hereditary Persistence of Fetal Haemoglobin
HPLC	High Performance Liquid Chromatography
IEF	Isoelectric focusing
MNH	Muhimbili National Hospital
MoHSW	Ministry of Health and Social Welfare
MUHAS	Muhimbili University of Health and Allied Sciences
PCR	Polymerase Chain Reaction
RSTMH	The Royal Society of Tropical Medicine and Hygiene
SCA	Sickle Cell Anaemia
SCD	Sickle Cell Disease
SPSS	Statistical Programme For Social Sciences

1. INTRODUCTION

The aim of a neonatal screening programme is to identify those individuals within a population who have a specific serious disorder, the outcome of which can be ameliorated by early intervention.[1,2] Implementation of a generalized screening programme for neonatal diseases like abnormal haemoglobins should follow established principles such as the disease severity, identification of status at an early stage, amenable to an effective treatment and detectable with a non-expensive and widely applicable test; and has to be of significant public health challenges. Subjects detected with positive results should be offered an appropriate and effective treatment or prevention.[3]

One of the conditions like Sickle cell disease (SCD) is the commonest abnormal haemoglobins associated with significant morbidity and mortality particularly in undiagnosed infants presenting for urgent care with their first sickle crisis. Neonatal screening programmes for abnormal haemoglobins have been reported as an important stage in detection of all the clinically significant conditions for which an early intervention is likely to be beneficial for those who are significantly affected and those who are carriers.[4-8] If associated with clinical care programmes, they have proven to be an important measure at decreasing mortality and morbidity in many regions, as well as providing counselling services to parents in preventing SCD.

Neonatal screening programmes for abnormal haemoglobins include identification of relevant haemoglobin (Hb) variants such as HbSS, HbSC, HbSD-Punjab, HbS/β-thalassaemia (β+,β°,∂β, Lepore), HbSO-Arab, and HbS/HPFH.[8,9] In addition to these sickle cell disorders, other conditions that could be detected at birth and which could be beneficial to the patient include; β-thalassaemia major, β-thalassaemia intermedia, alpha thalassaemia, HbE/β-thalassaemia, and HbS/E.[8,9] Successful programmes included simple methods for mass screening like isoelectric focusing (IEF) on cord blood or eluates of dried blood spots. Other programmes perform High Performance Liquid Chromatography (HPLC) as the initial screening method, which has the advantage that it can detect small proportions of variant haemoglobins. Following

initial testing, confirmation of abnormal specimens is done with a second electrophoretic technique, HPLC, immunologic tests, or DNA-based assays as confirmatory procedures.[2,4,7,10]

In Europe and North America, systematic reviews of screening for abnormal haemoglobins recommended the implementation of both antenatal and neonatal screening programme for abnormal haemoglobins and sickle cell disease as effective and appropriate screening programmes.[2,5,11]

Similar neonatal screening approach for abnormal haemoglobins has been reported in various American and African countries; however no similar screening programme that has been reported in Tanzania.

2. BACKGROUND

Haemoglobinopathies are genetic globin gene disorders, characterized by the presence of variant haemoglobin or a decreased or absence of globin chain synthesis, known as thalassaemia.[12-14] Sickle cell disease (SCD) is an inherited chronic haemolytic anaemia whose clinical manifestations arise from the tendency of the haemoglobin (HbS or sickle haemoglobin) to polymerize and deform red blood cells into the characteristic sickle shapes. This property is due to a single nucleotide change in the β-globin gene leading to substitution of valine for glutamic acid at position 6 of the β-globin chain ($\beta^{6glu\rightarrow val}$ or β^{s}).[10,15-17] The term sickle cell disease is therefore used to denote all entities associated with sickling of haemoglobin within red blood cells and that severity of sickling disorders varies among affected individuals.

The homozygous state (SCD-SS or sickle cell anaemia) is the most common and severe haemoglobinopathy in African populations, but interaction of HbS with beta thalassaemia and certain other variant haemoglobins can also lead to sickling in the red blood cells.[10,15,18] The haemoglobin S gene has a high frequency among persons of Africans ancestry and in India. It is also seen in the Mediterranean and Near East including Saudi Ariabia.[18] The clinical features of SCD-SS include increased infection

risk, a variable degrees of haemolysis leading to anaemia, and intermittent episodes of vaso-occlusion that cause tissue ischemia, leading to pain, acute and chronic organs dysfunction. Heterozygous individuals have a sickle cell trait (HbAS), a generally benign, asymptomatic carrier state.[18-22]

Sickle cell diseases are inherited in co-dominant recessive types as an autosomal recessive disorder. In most cases the parents of a child with a sickle cell disease are usually unaffected, healthy carriers of the condition and have one normal gene and one abnormal gene.[14] With each pregnancy, carrier parents have a 25 percent chance of having a child with two copies of the abnormal gene (SS), resulting in a haemoglobinopathy. Carrier parents have a 50 percent chance of having a child who is an unaffected carrier (AS) and a 25 percent chance of having an unaffected, non-carrier child (AA). These risks hold true for each pregnancy.[14] SCD-SS therefore occurs when one gene for production of HbS is inherited from each parent.

Other sickling disorders occur when the child inherits a gene for HbS from one parent and a gene for another abnormal form of haemoglobin (C, E or beta thalassaemia) from the other parent.[14] HbC is a variant haemoglobin in which lysine replaces glutamic acid at the sixth amino acid position of the β-globin chain.[15] Various genetic and environmental factors, found to contribute to the heterogeneity of SCD, include beta-globin haplotypes alpha thalassaemia and persistence of high fetal haemoglobin levels (HPFH).[23] The Bantu haplotype, commonly found in East Africa, is associated with severe disease as it is associated with a low level of HbF compared with other haplotypes.[24]

Thalassaemia is a genetic defect that results in decreased production of particular chains. This may affect the α, β, γ, or δ chains, or may affect some combination of the β γ, and δ chain in the same patient.[15,25] The result is an imbalance in production of globin chains and the production of hypochromic and microcytic RBCs that contain a surfeit of the unaffected chains which cannot combine stoichiometrically with the inadequate supply of thalassaemia chains.[15,25] These unmatched chains in turn can produce adverse effects on the red cell and lead to destruction of the red cell in the marrow (ineffective

erythropoiesis, a dominant feature of beta thalassaemia) and in the circulation (haemolysis, the dominant feature of alpha thalassaemia).

The α-thalassaemias occur widely throughout sub-Saharan Africa, the Mediterranean region, the Middle East, the Indian subcontinent and South-East Asia in a line stretching from southern China through Thailand, the Malay peninsula and Indonesia, to the Pacific island populations.[9] Because both HbA and HbF have α-chains, genetic disorders of α-chain synthesis result in defective foetal and adult haemoglobin production. In the foetus, a deficiency of α-chains leads to the production of excess γ-chains, which form γ4-tetramers, or Hb-Bart's; in adults, excess of β-chains form β4-tetramers or HbH.[9] Since there are two α-globins genes on chromosome 16, two main types of α-thalassaemia exists: α^0- thalassaemia, in which both genes are inactivated (– –/αα), and α^+- thalassaemia, in which only one of the pair is affected (–α/αα).[9]

There are two important clinical disorders caused by α-thalassaemia, the Hb Bart's hydrops syndrome which results from the homozygous inheritance of α^0-thalassaemia (– –/– –) and HbH disease which usually results from the co-inheritance of both α^0 and α^+-thalassaemia (–α/–) hence the former is incompatible with uterine life while the other condition manifest during adulthood.[9]

Several distinct β-globin gene haplotypes are associated with the sickle mutation, and their distribution provides evidence for origin of the mutation in several locations within Africa (the Senegal, Benin and Bantu haplotypes) and Asia (the Arab–Indian haplotypes).[2] The sickle trait bestows survival benefit in areas endemic for *Plasmodium falciparum* malaria, and the distribution of SCD historically paralleled this disease. The sickle haemoglobin containing red cells inhibit proliferation of *Plasmodium falciparum*, and are more likely to become deformed and removed from the circulation. In recent times, the dissemination of the sickle mutation in different areas of the world took place from the movement of populations via trade routes and the slave trade.[2,26]

3. REVIEW OF THE LITERATURE

The prevalence of abnormal haemoglobins varies tremendously among ethnic and tribal groups within a given geographical area. Sickle cell disease in particular is observed occasionally among the white population but has high frequency among Africans and African descendents, as well as in India, Greece, and the United States.[18] For example, 10% of patients with SCD-SS identified by the California newborn screening programme are not of African descent,[26] but also of Mediterranean, Caribbean, South and Central American, Arabian or East Indian heritage.[2,18,27-29] While SCD is not common in Western Europe, its increased frequency over the past 20 years was reported to be due to migration from African countries, thus leading to the development and application of successful neonatal screening programme in various European countries.[2,30]

About one out of every 951 African-American children is affected by sickle cell disease, making it one of the most prevalent genetic diseases in the United States.[17] In California (United States), a newborn haemoglobinopathy screening programme using IEF method on cord blood evaluated two million newborns and found 492 with SCD, while 290 had SCD-SS, 143 SCD-SC, and 47 SCD-Sβ^+-thalassaemia.[27] The screening programme implementation in that and other studies concluded neonatal screening to be an important step in increasing survival, reducing hospitalization, and minimizing expenses associated with sickle cell disease in US.[27-29]

A targeted screening programme in Canada using the cord blood of 9,619 infants born in Montreal Quebec whose mother or father was of African ancestry was collected at birth and analyzed for the presence of hemoglobin S by HPLC or IEF. Results show that 10.5% (n=1,012) had sickle cell trait and 4.0% (n=386) had HbC trait while 0.74% (n=72) of infants were diagnosed with SCD; about 0.4% (n=37) were classified as SCD-SS or SCD-β-thalassaemia and 0.4% (n=35) had SCD-SC.[4] That screening programme was reported as clinically effective because it identified 92.3% of at-risk neonates who received appropriate medical care before 10 weeks of age instead of at a median of 12 months for infants not identified by the screening programme in Canada.

High frequency of abnormal haemoglobins amongst newborns has been reported by several authors during the screening researches or implemented screening programmes. Studies in Brazil revealed a high prevalence of haemoglobin disorders; 6.6% sickle cell trait (HbAS) and 0.09% SCD (0.07 % SCD-SS and 0.02% SCD-SC) were observed.[31] These frequencies varied widely according to the degree of racial admixture in the different regions of the country. In Southern Brazil for example, the gene was found at a frequency of 1.2% in newborns. HbC has a prevalence of 3% among African-Americans in the US and 1-3% among Puerto Ricans. However in Brazil, depending on the region HbC carriers are 2.2% to 5.2% of the population.[31,32]

Other studies in Brazil have reported a prevalence of 0.1 to 0.3% of newborns with SCD-SS and 20.0 to 25.0% of heterozygous α^{+}-thalassaemia among African Brazilians.[33-35] In the North-East of Brazil, $-\alpha^{3.7}$deletion was investigated in 106 pregnant women with AC and AA phenotypes, showing a 21.7% heterozygous and 0.9% homozygous rate for this mutation.[14,33,34] The most recent cross-sectional epidemiological study analyzed 590 neonates delivered in Salvador, Brazil whereby umbilical cord blood samples were analyzed by HPLC and Gap-PCR to detect alpha thalassaemia.[14] Results of that study revealed that 9.8% (n=57) were FAS; 6.5% (n=36) FAC; 0.2% (n=1) SF; and 0.9% (n=5) were FSC. Nearly 22.2% (n=114) newborns had $-\alpha^{3.7}$ deletion, of whom 19.7% (n=101) were heterozygous and 2.5% (n=13) homozygous. It was concluded that frequencies found in that study confirmed that abnormal haemoglobins are a public health problem in Brazil, and hence emphasized the need for neonatal screening and genetic counseling programme to be in place.

Although the overall birth rate in Europe is lower, in recent decades there has been a rapid increase in the number of individuals with haemoglobin disorders, particularly in urban areas. In London for example, SCD is now one of the most common reasons for admission to hospital.[36,37] The provisional figures from the first seven months of (up to March 2004) 108,255 infants screened in England gave a screen positive rate of 1:900 for immigrants from high prevalence areas and a carrier rate of 2.7% using blood spot test on HPLC and IEF for the neonates at 5–8 days of age.[38] Within the same country, figures for 2004–2005 show about 250 significant screen positive results for sickle cell

disorders and about 6,500 carriers were identified.[38] The birth prevalence for screen positive results from 2004–05 is 1:1,500. Furthermore, authors estimated that when there is countrywide data, the national birth prevalence would be about 1:2,000. The results from that national newborn sickle cell screening programme in England show that the sickle cell disorders are as common as cystic fibrosis (CF) in England, although the distribution of cases is concentrated in London and other urban areas. The findings and approach to implementation adopted in England may be of interest to other Western European and also to other developed countries with increasing rates of SCD who are considering such programmes.[38]

In Spain, sickle cell disease was considered anecdotal until recently, when the diagnosis of this entity has markedly increased as a result of immigration. A prospective, descriptive study was designed to include the 190,238 neonates born in the autonomous community of Madrid. A heel prick dried blood spot was analyzed by HPLC to detect haemoglobins F, A, S, C, D and E. The results of the first 32 months of universal neonatal screening for abnormal haemoglobins detected 1,060 haemoglobin variants (5.57 for every 1000 births).[39] Thirty-one were SCD whereby appropriate antibiotics, vaccination and comprehensive care were initiated and prenatal diagnosis of subsequent pregnancies was performed in three families after parental investigation.

The universal screening programme for abnormal haemoglobins is expected to reduce morbidity and mortality in the first years of life. A study in Belgium explored the feasibility of systematic screening for the presence of haemoglobin S or C in the eluted blood examined by an enzyme-linked immunosorbent assay (ELISA) test performed with a monoclonal antibody. Among the 27,010 samples tested, 0.49% (n=132) were positive. Further confirmatory molecular testing performed on dried blood from positive samples showed that 106 of these infants were AS and three were AC, while 0.011% (n=3) of the newborns were SS.[5] In that study, 0.063% (n=17) of samples were false-positives as researchers could not detect any mutation; findings that added a new immunological approach in the field of haemoglobinopathy neonatal screening and proposed an extension in a wide range of countries, especially in Central Africa where there is high prevalence of abnormal haemoglobins.

Children affected with SCD are at increased risk for severe morbidity (for example severe hemolytic anaemia, splenic dysfunction, pain crises, and bacterial infections) and mortality, especially during the first five years of life. In Africa for example, the World Health Organization estimates that SCD causes 9% of deaths in children under five (and it is up to 15% in some countries in West Africa), where infection remains the main cause of death.[40] The incidence of SCD in West Africa, is relatively high and constitutes a major public health problem.[41,42] With improvements in health and nutrition conditions, West African infant mortality declines and genetic disorders emerge as important causes of deaths and chronic disabilities as exemplified in Burkina Faso whereby the gene frequencies for HbS and HbC in adults were estimated to be approximately 0.04 and 0.10, respectively.[43,44]

The feasibility of a neonatal screening programme as well as adaptations of tools that are locally cheap and acceptable invites a challenge in implementations of an effective and successful programme in a given setting. In Burkina Faso for an example, a pilot study to determine the incidence of SCD and the feasibility of a neonatal screening programme in 2000, 2003 and 2004 was done in Ouagadougou by using an isoelectric focusing technique from cord blood of newborns. Results from 2,341 samples screened showed that the incidence of SCD was 1:57; 14 neonates were SCD-SS and 27 were SCD-SC. Thirty-two neonates were reported CC, while the incidence of the HbC trait was 1:6; and incidence of the HbS trait was 1:14.[45] Authors of that study concluded that SCD should be considered a major public health problem in Ouagadougou and that neonatal screening programme should be implemented, but to be effective it requires strategies adapted to the local situation.

In Central Africa, the prevalence of abnormal haemoglobins appears high (1.65%) and more than half of the affected children die before the age of five years.[46] The survivors develop degenerative complications and experience increased morbidity and reduced life expectancy. However, early detection followed by precocious and specialized medical care reduces morbidity and mortality of these patients.[10,46] A study to evaluate the feasibility of systematic neonatal screening for sickle cell disease was conducted in the region of Great Lakes in Central Africa (Burundi, Rwanda and the East of the

Democratic Republic of Congo). Newborns were tested for the presence of haemoglobin C and S in the eluted blood by an enzyme-linked immunosorbent assay (ELISA) test using a monoclonal antibody. Of the 1,825 samples screened, 5.32% (n=97) were positive. Of these, 3.28% (n=60) of samples were AS; 0.22% (n=4) for AC; and 0.11% (n=2) of the newborns were SCD-SS.[10] ELISA tests were found appropriate for developing countries due to its lower cost and the high specificity: such systematic screening for SCD was therefore recommended to be feasible.

Despite the widespread use of neonatal screening programmes for SCD in Europe and the Americans, few studies have focused on the neonatal screening for abnormal haemoglobins in sub-Sahara African countries. Tshilolo and colleagues[47] evaluated the need for a neonatal screening programme for SCD, and proposed a realistic health care programme for newborns with SCD in those countries, based on personal experiences in DRC-Kinshasa and Burkina Faso. In that review, well-established criteria for the development of neonatal screening programmes for SCD in sub-Sahara African countries were recommended. It included all regions where the incidence of the disease is 0.5 per 1,000 or higher, and those newborns that have an AS or AC mother. Furthermore, a need for SCD clinical care programme which includes infection prophylaxis with penicillin and anti-malarials should be implemented as well.[47]

A prospective, observational study was established at Muhimbili (MUHAS and MNH) in Dar-es-Salaam Tanzania between 2003 and 2007. Amongst other objectives, one was to calculate the expected prevalence of people with SCD-SS in Tanzania applying an indirect method using the Hardy-Weinberg equation that involves prevalence of AS and country population. The prevalence of the heterozygous carriers (AS) of sickle gene was determined by cross sectional surveys of blood donors at MNH by cellulose acetate electrophoresis at pH 8.2 (Helena Biosciences) while demographic information was obtained from the year 2002 population and housing census country report. A preliminary report using this indirect method to calculate the expected birth rate of SCD-SS, estimated that there will be up to 14,000 children born every year with SCD in Tanzania.[48] Unfortunately, the absence of a neonatal screening programme in

Tanzania led the authors to predict that, the majority of SCD children may die even before the diagnosis and relevant intervention is achieved.

4. PROBLEM STATEMENT

Abnormal haemoglobins are reported as a significant global public health issue. Some publications report that haemoglobin disorders (sickle cell and alpha and beta thalassaemia) are the world's most common genetic defects with annual estimates of 300,000–500,000 births (83% sickle cell disorders, 17% thalassaemia) across 193 countries.[49,50] A World Health Organization[51] report on global epidemiology of haemoglobin disorders and derived service indicators showed that haemoglobin disorders present a significant health problem in 71% of 229 countries, and these countries include 89% of all affected births worldwide.

Sickle cell disease is a common monogenic disorder and represents a largely unrecognized health problem for millions of Africans, and has been identified as a condition of increasing public health significance.[52] It is estimated that SCD affects three of every 1000 African newborns and approximately 50,000 persons of African ancestry in the United States.[21] Reports from UK suggested that more than 10,000 people and more than 600 would have SCD and Beta Thalassaemia by the year 2000 respectively.[2]

In sub-Saharan Africa, the prevalence of SCD is reported as the highest worldwide. The prevalence of sickle cell gene carriers varies between 5% and 40% of the population, and most children die in early childhood without being diagnosed or because treatment protocols have not been adapted to the local situation.[53] The estimated birth prevalence of sickle cell disorders in Africa ranges from 7 – 15 in every 1000 live births, while it is estimated that 8-14% of infant mortality (death in the first year of life) is potentially contributed by haemoglobin disorders.[54]

Some studies reported that children affected with SCD are at increased risk for morbidity in which *Streptococcus pneumoniae* is reported as the commonest cause of infections in these patients especially during the first three years of life, [21,22] and that early diagnosis of sickle cell disease could alert clinicians to potential clinical complications of the disease, and hence allowing prompt clinical care and prophylactic therapy with antibiotics and vaccines.[10,55]

The geographical distribution of SCD correlates with areas where *Plasmodium falciparum* is historically endemic, as they are naturally selected for by malaria. Modiano and other colleagues[43] have demonstrated that HbS and HbC are associated with a reduced risk of severe malaria. Nevertheless, if there is a limited sickle cell pathology associated with AC, AS and CC phenotypes, there is a severe disadvantage for SS and SC phenotypes.

Although malaria is endemic in Tanzania - an Eastern African country, there is no neonatal screening programme even though SCD is recognized as a public health problem, with the prevalence of people with the carrier state ranging between 15 – 20% and the estimated birth incidence of children with SCD-SS in Tanzania between 6 to 7 per 1000 children.[48] These figures imply that within the next 5 years there will be over 60,000 children born with SCD and the majority of these children will not know that they have SCD unless neonatal screening programmes are implemented.

5. RATIONALE

The objective of this study was to establish the frequency and types of haemoglobin variants in Tanzania since there has never been a previous neonatal screening programme in the country. The screening programmes implementation is an important step for increasing survival, reducing hospitalization, and minimizing expenses associated with sickle cell disease.[10] If a newborn is screened and diagnosed with SCD at birth, preventive treatment like pneumococcal prophylaxis and meningococcal immunizations can be started within the first two to three months of life to save a child from devastating complications and death.[3-8]

The earlier the neonate is screened and diagnosed with abnormal haemoglobins, the sooner parents can be educated about what they need to do for their child [6,43] and that, screening does permit genetic counseling for parents with affected or carrier newborns and especially when the parents are contemplating more children.[11]

At present, only a few countries in sub-Saharan Africa have published their experience of a comprehensive clinical care including neonatal screening programme for abnormal haemoglobins especially SCD with the possibility of introducing such programmes free of charge.[56] This lack of SCD integration in the global health priorities is incomprehensible. For many years, in most countries in sub-Saharan Africa, malaria, respiratory tract infections, gastro-intestinal infections, parasitological infections and human immunodeficiency virus have been recognized as health priorities, but this is not the case for SCD and related abnormal haemoglobins.

This study not only assisted for early diagnosis of neonates with SCD, but also carriers of S gene and other abnormal haemoglobins (Delta chain variant, β-thalassaemia, and Hb Barts – ά thalassaemia) that were detected at birth; and hence provided an opportunity for preventive measures (genetic counselling to parents of affected neonates) and planning for early treatment of anticipated complications.

6. STUDY OBJECTIVES

6.1 Broad objective

To determine the frequency and types of abnormal haemoglobins in neonates at Muhimbili National Hospital, Dar es Salaam, Tanzania

6.2 Specific objectives

6.2.1 To determine the frequency of occurrence of abnormal haemoglobins amongst neonates attended at MNH

6.2.2 To determine different types of abnormal haemoglobins amongst neonates attended at MNH

6.2.3 To determine factors that are associated with neonates with abnormal haemoglobins at MNH

6.2.4 To describe the haematological parameters (complete blood count) of neonates at MNH

7. METHODOLOGY

7.1 The study area

This study was conducted at the Muhimbili National Hospital (MNH), which is located in Dar es Salaam City, Tanzania. It is a tertiary, referral and teaching hospital with a service delivery system that serves the whole country. The Department of Haematology and Blood Transfusion where the study was done, is among the clinical and laboratory Departments at MNH that provides diagnostic and management services for persons with a wide range of haematological disorders including anaemia, bleeding disorders, malignancies, abnormal haemoglobins and blood transfusion services. In addition to outpatients' clinic services provided for adults and children, routine in patient care is provided to such patients admitted to MNH.

In recognizing the importance of neonatal screening and comprehensive care for children with SCD, the Department of Haematology developed an integrated system at MNH which acts as the national centre for diagnosis and provision of comprehensive care and management to patients with SCD. Over the past 3 years, a cohort of 2,000 individuals with SCD has been established.

Dar es Salaam is Tanzania's largest city and one of the commercial centres of East Africa. It is divided into three administrative municipalities namely Ilala where MNH is located, Temeke and Kinondoni. The population has grown rapidly from around 450,000 in 1967 to 2,497,940 at the last census in 2002, [57] however the population is currently estimated to be nearly 5 million, while the total country population is estimated to be nearly 40 million within 26 administrative regions.[57]

7.2 Study design

This was a hospital based descriptive cross-sectional study in which quantitative data collection method was applied.

7.3 A Study population description

7.3.1 Study participants

The study enrolled neonates who were receiving services at MNH during the time of data collection regardless of being delivered in or outside this hospital. The participants were found in neonatal and postnatal wards.

7.3.2 Selection criteria

Inclusion criteria:

i) Neonates (28 days of age and below)

ii) Informed consent from a parent

iii) Participants who had no history of blood transfusion at a time of data collection

Exclusion criteria:

i) Infants of more than 28 days of age

iv) Parental refusal to allow her/his neonate to participate in the study

ii) Participants who had history of blood transfusion at a time of data collection. (If the neonate has been transfused, then the test was done but the results not entered on the study. This is because results from transfused neonates are not reliable to exclude SCD. Any child found to have abnormal results was offered test after three months).

7.4 Sample size estimation

Preliminary report in an unpublished study by Makani and colleagues in Dar es Salaam has reported a carrier rate for SCD of 15% - 20% in the general population.[48] The sample size estimation for this study was based on the assumptions that;

- The rate of abnormal haemoglobins (P) amongst neonates was 15%
- The critical value (Z) was (1.96) and
- Marginal error (ε) was estimated at 2%.

Using the EPIINFO 6.04 programme,[58] sample size (n) was calculated by the formula:

- $n = \frac{Z^2P(1-P)}{\varepsilon^2}$

From the calculation, the minimum sample size required for this study was 1200.

Contrary to hospital records from September to December in 2008 from which the preliminary assumptions for availability of study population (450 per month) were made,[59] there were a total of 2,179 neonates who were available for study participation during the three months period of data collection. Since resources for this study allowed for recruitment of a larger than the calculated sample size, a total of 2,108 neonates who met eligibility criteria for inclusion into study were therefore recruited.

7.5 Sampling procedure

A list of all neonates in neonatal and postnatal wards was checked by using a hospital electronic data base on daily basis. Their case files were reviewed to identify neonates who met the inclusion criteria. The list was further refined to include only neonates who met the inclusion criteria to participate and thereafter, consecutive parents of the participants were approached on the same day for informed consent.

7.6 Data collection instruments and procedures

7.6.1 Instrument proforma questionnaire

A structured interview proforma translated into Kiswahili (the language easily understood in this setting by both interviewer and interviewee) with identification (ID) number was administered to the parents of all eligible participants. Information on demographic variables such as age, sex, blood transfusion status, birth weight and gestation age at delivery were gathered. Additional information such as, marital status and geographical origins of their parents, was collected too *(Appendix 4)*.

7.6.2 A registration log book

A registration log book was used to summarize information on the participant's ID number, hospital file number and his/her initials. Other information captured included name of respondent, the names of parents, their telephone numbers, map cues of their residence, date of enrolment, and initials of the researcher/research assistant who attended the participant. The recruitment log book was kept separately from the questionnaires so as to maintain confidentiality of data obtained, provide tracing information for quality assurance of data collection, and was used to track down the

participants for the results feedback when they had already left the hospital prior to receiving results *(Appendix 3)*.

7.6.3 Data collection procedure

The main researcher and research assistants provided informed consent to respondents, administered structured interview proforma and collected blood specimen from participants, labelled and sent them to laboratory for analysis. Filled forms were checked daily during supervision sessions with the study team to ensure good data quality.

7.6.4 Specimen collection

Blood specimens were drawn using aseptic techniques for assessments of abnormal haemoglobins and haematological parameters (CBC) in a place where a participant was attended (postnatal or neonatal ward). Heel prick blood samples of approximately 250 µL were collected on ethylene diamine tetra-acetic acid (EDTA) Teklab 500 µL with microgard closure tubes. Thereafter samples were appropriately labeled using ID number corresponding to ID questionnaire's number, date of collection and then were transported to Central Pathology Laboratory (SCD project section) for analysis.

7.6.5 Laboratory procedures

Specimens for complete blood counts (CBC) were placed at room temperature and analyzed within 8 hours of collection, thereafter they were refrigerated at $2^{o}C$ to $8^{o}C$ waiting for sufficient number of samples (minimum of 89 samples, and before 7 days) for abnormal haemoglobins analysis by HPLC.

Complete blood counts were performed to all sufficient and non clotted study participants' samples using ABX PENTRA 60 (ABX Diagnostics, Irvine, CA, USA). Parameters assessed were; Hb level, red blood cell (RBC) count, haematocrit, mean corpuscular volume (MCV), mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), red cell distribution width (RDW), platelet counts, white blood cell count (WBC) including differentials.

(Note: Haematology analyzer does not distinguish nucleated RBC from WBC).

High performance liquid chromatography (HPLC); using the β-thalassaemia short programme on a Bio-Rad variant *(Bio-Rad, Laboratories, Inc. Hercules, CA, USA)* test was used to determine the presence of fractions of haemoglobins in whole blood. HPLC has been effective in detecting small quantities of abnormal haemoglobin variants, and abnormal amounts of normal haemoglobins. It is reported to be a sensitive, efficient, and time saving, depending on the genotype alternative to IEF for neonatal screening of common abnormal haemoglobins,[60] and have shown a sensitivity and specificity of 0.9945 and 0.9999 respectively as well as positive and negative predictive values of 0.9978 and 0.9998 respectively.[61]

Unaffected full term neonates were found to have mostly foetal hemoglobin (HbF) and some adult hemoglobin (HbA). An infant testing positive for any form of SCD was found to have HbF with HbS and depending on the genotype abnormal hemoglobin such as HbD-variant or beta thalassaemia. All abnormal neonates screening test results indicating the presence of HbS underwent further second line (confirmatory) test using haemoglobin electrophoresis (cellulose acetate electrophoresis at pH 8.2 (Helena Biosciences). All the tests above were performed in the SCD laboratory situated at Central Pathology Laboratory of MNH. The results were interpreted by the researcher and discussed with her supervisor who is a specialist laboratory scientist and visiting lecturer from England.

7.7 Preparations for Study

7.7.1 Research assistants' recruitment

Seven clinical research assistants (holders of Diploma in Nursing) experienced in working with neonates and with previous research experience were recruited and trained. The training covered the purpose of the study, data collection techniques, familiarity with the data collection tools and strategies for data quality assurance. A laboratory technologist who is familiar with HPLC, Hb electrophoresis techniques and haematological analyzer machine was recruited for specimen analysis and recording of results. A data entry clerk was also recruited for electronic data entry in a computer ready for analysis. The researcher's roles in this study included training of research assistants, assisting in data collection, interpretation of haematological, Hb

electrophoresis and HPLC results as well as supervision of the study team to ensure good quality of data captured.

7.7.2 Pilot study

A pilot study was conducted amongst 20 neonates. The aim of the pilot was to train the research assistants in the use of the checklist to select eligible neonates, to ensure the research assistants knowledge on how to collect the relevant neonatal data and to train the research assistants in the techniques used to collect heel prick samples. Any encountered problems were addressed, and amendments of the questionnaire and research procedures were considered when deemed necessary.

7.8 Data analysis

Data entry was done by ACCESS programme in Microsoft office 2007, and then exported to SPSS statistical programme version 16 for analysis.[62] Data double entry was done to ensure data validity. Continuous data were presented as mean, standard deviation and percentage while chi square (X^2) test was used to compare categorical data, and the statistical significance level was considered when p-value was ≤ 0.05.

7.8.1 Haemoglobin analysis

Haemoglobin disorders were dichotomized as normal or abnormal haemoglobin. Further confirmatory test of positive results (Hb electrophoresis) reported frequencies of occurrence of abnormal haemoglobins, as well as distinguishing carriers from those with SCD. Cross tabulation with chi square statistics and p values at 95% confidence intervals were used to assess associations of occurrence of abnormal haemoglobins with non continuous demographic characteristics and haematological parameters collected in this study.

7.8.2 Demographic characteristics

During the analysis of demographic variables, age in days was grouped into four categories; first week (1 – 7 days), second week (8 – 14 days), third week (15 – 21 days), and fourth week of life (22 – 28 days). Sex was dichotomized as male and female; birth weight in kilograms was categorized as low birth weight (< 2.5kg),

normal birth weight (2.5 – 3.5 kg) and high birth weight (above 3.5kg). Gestation age was determined as per expected date of delivery and expressed in weeks; premature (< 37 weeks), mature (37 – 40 weeks), and post mature (above 40 weeks). Geographical origins of participants' parents were recorded according to government recognized regions and later categorized by nationally recognized zones.

7.8.3 Haematological parameters

Analyses of haematological parameters (CBC) were reported as frequency of occurrence, mean and standard deviation from the study participants.

7.8.4 Describing operational factors

Operational factors identified during the three months period of data collection were systematically recorded in a log book. It included problems and challenges identified during the recruitment, administration of questionnaires, sample collection, sample analysis, giving feedback results to parents, and clinical care of neonates who were identified to need urgent treatment. Information gathered was further categorized into various factors, summarized and presented as a table *(Appendix 5)*.

8. ETHICAL CONSIDERATIONS

Prior to recruiting participants, their parents were invited to an informed consent process by the principal researcher and/or her recruited clinical research assistants. The invitation to participate was given in a designated side rooms available in each postnatal wards. The purpose of the study was described, study procedures, risks, benefits and alternatives to participation *(Appendix 1)*. Information on how parents with questions related to the study could access the principal investigator and also information on how they could access the chairperson of the Senate University Research and Publications Committee should they wished to ask for their rights of study participation were also provided. Willing parents were required to provide signed consent witnessed by researcher/research assistants *(Appendix 2)*. The voluntary nature of study participation was explained to each potential participant's parent and that

refusal to participate would not in no way affect the participant's rights to standard treatment at the hospital. The alternative to participation was provided as not to participate.

Soon after the screening results were available, feedback information was given to all the parents of the participants. In case they had left hospital (discharged home) before results were given out, information gathered from a registration log book were used to track them down. For those identified to have clinically significant abnormal haemoglobins and other detected haematological disorders, arrangements were made between physicians (paediatricians and haematologists) in charge of the affected neonates for further appropriate medical care whereby homozygous patients received relevant comprehensive preventive measures as well as genetic counselling to parents for future pregnancies. The parents of the heterozygous neonates were also contacted for further information and appropriate measures to follow through. Neonates with any other detected haematological disorders were also been identified and engaged to treatment options available under responsible physicians. These included neonates with significantly reduced haemoglobin level at birth where transfusion was indicated.

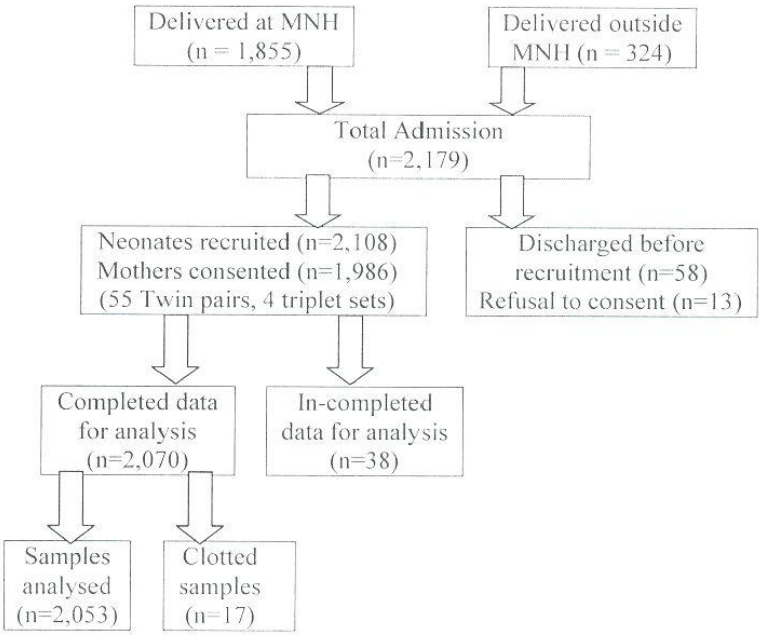
Measures to ensure confidentiality of the information provided were addressed. They included absence of names on all forms (ID numbers only were used) and safe handling/keeping of data collected by the researcher alone until the end of analysis. All data records are kept for future review and will be utilized only for the purposes stated.

No data collection preceded ethical clearance to conduct the study. Ethical clearance for the study was attained from the chairperson of the Senate University Research and Publications Committee of MUHAS, and permission was also granted by the director of clinical services from MNH management.

9. RESULTS

A total of 2,179 neonates were admitted into postnatal and neonatal wards of MNH during the time of data collection from 1st September 2009 to 30th November 2009 *(Figure 1)*. Of these, 85.3% (1,855/2,179) were delivered at MNH and the reminder presented to the neonatal ward after delivery due to neonatal or maternal illness. The overwhelming majority (2,108/2,179) of the study participants were from 1,986 mothers who were invited to the study and provided consent for participation giving a response rate for eligible study participants of 96.7% (there were 4 sets of triplets, and 55 pairs of twins). Samples from 98.2% (2,070/2,108) participants whose mothers consented were collected for laboratory processing and data analysis. However 97.4% (2,053/2,070) of samples were collected and successfully analysed. The reasons for missing data are outlined (*Appendix 5*) as part of the describing operational factors that intended to inform the logistics and operational issues of this study in the forthcoming cohort looking at the same objective but will have also concurrent intervention programme.

Figure 1: Flow chart procedure during study period (September - November 2009)



9.1 Frequency of occurrence of abnormal haemoglobins

HPLC was used to determine the frequency of neonatal abnormal haemoglobins at MNH and was successful on 2,053 samples. The results showed that 18.2% (n=374) neonates participated in this study had abnormal haemoglobins *(Table 3)*. Other findings are summarized in the following sub-sections.

9.2 Haemoglobins identified in the study

In this study, five types of haemoglobins were identified. *Table 1* summarizes the different types of haemoglobins found during HPLC analysis. The majority of the study population 81.8% (n=1,679) were Hb FA (normal). The abnormal haemoglobins identified were Hb FAS (sickle cell trait) 12.6% (n=258); Hb Barts 5.3% (n=109); Hb FS (SCA or Hb S Beta^0 -thalassaemia) or Hb S with HPFH 0.54% (n=11); Hb FSA (sickle cell carrier or HbS Beta $^+$ -thalassaemia) 0.9% (n=19); and one participant had Hb FA and D variant. The rate of sickle gene carrier (FAS, FS and FSA) in this result is calculated as 14% (n=288).

Table 1: Types of haemoglobins **N=2053**

Phenotypes	Interpretation	N	%
FAS**	Sickle cell trait(carrier)	258	12.57
FS**	Sickle cell anaemia, OR Sickle cell- Beta ^{0} – Thalasaemia	11	0.54
Hb-BARTS *	Haemoglobin Barts (α-Thalasaemia)	85	4.14
FAD	Haemoglobin D-variant Carrier	1	0.05
FA	Normal Haemoglobin	1679	81.80
FSA**	Sickle cell trait(carrier) OR Sickle cell-Beta$^+$ -Thalasaemia	19	0.93

** A total of 109 Hb Barts were identified. Out of these, 22.0% (n=24) were compound heterozygous with FAS. Therefore Hb Barts in this table is recorded as only 85.*

*** Sickle gene carrier rate in this table sum up to 14% (n=288).*

9.3 Hb Electrophoresis results

After the frequency and types of abnormal haemoglobins were identified by HPLC, further confirmatory tests were done to 94.5% (n=273/289) of samples with SCD and D variant using Hb electrophoresis as per protocol. *Table 2* shows the results of electrophoresis in comparisons to results of HPLC. Out of 11 participants who showed the presence of haemoglobin FS on HPLC, only 27% (n=3) exemplified the same results on Hb Electrophoresis, and 9% (n=1) showed only haemoglobin F. Further, out of 109 Hb Barts identified by HPLC, only 30% (n=33 Barts) from FA + Barts (n=6); FSA + Barts, (n=1) and FAS + Barts (n=26) were identified by Hb electrophoresis. While Hb electrophoresis was not able to pick up the Hb D variant which was identified by HPLC, the confirmatory test could not distinguish FAS from FSA or FAD and rather showed them as haemoglobins A, F and S.

Table 2: Hb Electrophoresis results following HPLC analysis N=2053

	(HPLC RESULTS)						
	FS* n (%)	**FAS* n (%)**	**FA n (%)**	**Hb Barts n (%)**	**FSA* n (%)**	**FAD* n (%)**	**Total N (%)**
	N=11	N=258	N=1679	N=85	N=19	N=1	
Hb Electro phoresis Results							
FAS	7 (63.6)	156 (60.0)	1 (0.1)	6 (7.10)	9 (50.0)	-	1760 (85.7)
F	1 (9.1)	9 (3.5)	-	-	-	-	10 (0.5)
FA	-	48 (18.5)	2 (0.1)	3 (3.50)	5 (26.3)	1 (100)	56 (2.7)
FA+BARTS	-	6 (2.3)	-	1(1.20)	-	-	6 (0.3)
FAS+BARTS	-	26 (10.0)	-	4 (4.70)	1(5.6)	-	27 (1.3)
FS	3 (27.3)	3 (1.2)	-	-	-	-	6 (0.3)
Hb Eletro-phoresis NOT done	-	12 (4.6)	-	-	4 (22.2)	-	16 (0.8)

**Sickle cell disease and D variant sum up to N=289*

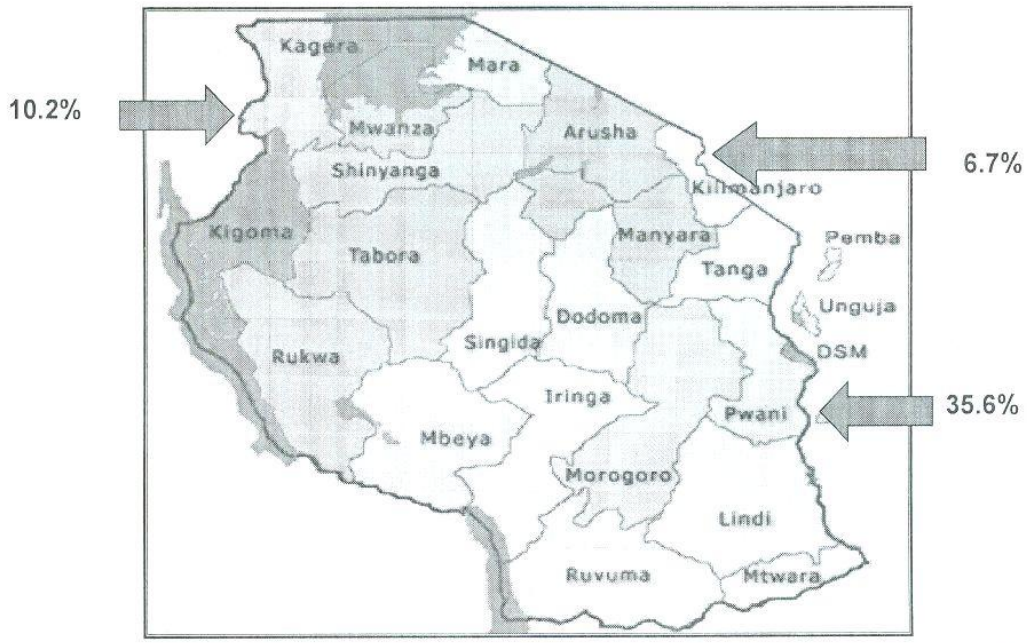
9.4 Demographic characteristics of the study population

The demographic characteristics of 2,053 participants were dichotomised according to their haemoglobin status as normal or abnormal *(Table 3)*. The age of the participants ranged from 1 to 28 days, (mean age = 1.2 ± 0.5 days), with 90.6% (n=1,859) of participants in the age group 1 to 7 days while 55.4% (n=1,138) were males. Nearly a third of participants 31.30% (n=643) resided in Kinondoni municipal, another 30.0% (n= 615) were from Ilala and the largest group 36.1% (n=741) reported to be residents of Temeke *(Figure 2)*. Nearly two thirds of study population 58.4% (n=1,198) had birth weight within normal range (2.5 to 3.5kg) and just over four fifths of them 82.30% (n=1,690) were delivered at term gestation age. A large proportion of parental origins of participants were noted to originate from Coastal Region 29.7% (n=610) while a minor proportion 3.1% (n=64) reported to originate from the Western part of Tanzania.

9.5 Demographic characteristic and haemoglobin types

Statistical analysis *(Table 3)* revealed two groups which were associated with having abnormal haemoglobins namely, neonates born pre-term, and neonates with parents from particular regions of Tanzania. Having abnormal haemoglobins increased the likelihood of neonates being born at low gestational age (23.8%) by 1.5 fold as compared to neonates (16.3%) born without abnormal haemoglobins (X^2=11.7, p=0.001). There is also 1.5 fold increased chances of abnormal haemoglobins amongst neonates whose parents originated from Lake Zone (10.2%) and 1.2 fold increased chances for those who reported to originate from the Coastal (35.6%) Regions (X^2=37.7, p=0.001). The same statistical analysis showed that neonates whose parents originated from Northern Regions of Tanzania (6.7%) had 2.5 fold low chances of having abnormal haemoglobins as compared to (16.9%) who were normal *(Figure 3)*. The association between abnormal haemoglobins with gestation age and origins of parents were the only demographic factors found to be statistically significant (X^2>1.96, p<0.01).

Figure 2: Map of Tanzania showing the frequency of distribution of abnormal haemoglobins



Lake zone includes Kagera, Mwanza and Mara; Coastal region is comprised of Tanga, Dar es Salaam, Pwani, Lindi and Mtwara; while Northern region is composed of Arusha, Kilimanjaro and Manyara

9.6 The association between SCD and demographic characteristics

Table 4 shows that the age, sex, birth weight and residence of study participants had no statistical significant relationship with SCD ($p>0.05$). However, the probability of inheriting sickle cell trait (FAS/FSA) was highest among participants whose parents originated from Lake Zone (10.1%) and Coastal Regions 36.7% by 1.5 and 1.3 fold respectively as compared to those with normal haemoglobins; while the probability of sickle cell trait was lowest (6.8%) by 2.4 fold amongst participants whose parents were originating from Northern Region ($X^2=44.7$, $p=0.01$). The probability of pre-term delivery was also found to increase by 1.5 fold amongst neonates with SCD. ($X^2=10.3$, $P=0.04$)

Table 3: Characteristics of the study population

	Abnormal haemoglobins n (%) N = 374	Normal Haemoglobin n (%) N = 1679	Total N (%) N = 2053	X^2 (p-value)
Age groups (days)				
01 – 07	339 (90.60)	1520 (90.50)	1859 (90.60)	
08 – 14	18 (4.80)	86 (5.10)	104 (5.10)	
15 – 21	12 (3.20)	46 (2.70)	58 (2.80)	
22 – 28	5 (1.30)	27 (1.60)	32 (1.60)	0.44 (0.93)
Sex				
Males	217 (58.00)	921 (54.80)	1138 (55.40)	
Females	157 (42.00)	758 (45.20)	915 (44.60)	1.24 (0.27)
Birth Weight (kg)				
< 2.5	113 (30.20)	417 (24.80)	530 (25.80)	
2.5 – 3.5	207 (55.30)	991 (59.00)	1198 (58.40)	
>3.5	54 (14.40)	271 (16.10)	325 (15.80)	4.69 (0.10)
Gestation (weeks)				
< 37	89 (23.80)	274 (16.30)	363 (17.70)	
37 – 40	285 (76.20)	1405 (83.70)	1690 (82.30)	11.75 (0.001)
Residence				
Temeke	145 (38.80)	596 (35.50)	741 (36.10)	
Kinondoni	104 (27.80)	539 (32.10)	643 (31.30)	
Ilala	111 (29.70)	504 (30.00)	615 (30.00)	
Outside Dar	14 (3.70)	40 (2.40)	54 (2.60)	4.88 (0.18)
Parental Origin				
Coastal	133 (35.60)	477 (28.40)	610 (29.70)	
Lake	38 (10.20)	113 (6.70)	151 (7.40)	
Central	83 (22.20)	331 (19.70)	414 (20.20)	
Northern	25 (6.70)	283 (16.90)	308 (15.00)	
Southern	60 (16.00)	349 (20.80)	409 (19.90)	
Western	16 (4.30)	48 (2.80)	64 (3.10)	
Zanzibar	19 (5.10)	78 (4.60)	97 (4.70)	37.73 (0.001)

X^2 = Chi square

Table 4: The association between SCD and demographic characteristics N=2052*

	HBFS (N=11)	HBFAS/FSA (N=278)	HBFA** (N=1763)	X^2 (P-value)
Age groups (days)				
01 – 07	9 (81.80)	252 (90.60)	1597 (90.60)	
08 – 14	-	14 (5.00)	90 (5.10)	
15 – 21	1 (9.10)	8 (2.90)	49 (2.80)	
22 – 28	1 (9.10)	4 (1.50)	27 (1.50)	8.30 (0.76)
Sex				
Males	5 (45.50)	170 (61.20)	962 (54.60)	
Females	6 (54.50)	108 (38.80)	801 (45.40)	9.29 (0.054)
Birth Weight (kg)				
< 2.5	1 (9.10)	80 (28.80)	449 (25.50)	
2.5 – 3.5	10 (90.90)	152 (54.70)	1035 (58.70)	
>3.5	-	46 (16.50)	279 (15.80)	11.69 (0.16)
Gestation (weeks)				
< 37	3 (27.30)	67 (24.10)	293 (16.60)	
37 – 40	8 (72.70)	211 (75.90)	1470 (83.40)	10.33 (0.04)
Residence				
Temeke	5 (45.50	112 (40.30)	664 (35.40)	
Kinondoni	3 (27.30)	76 (27.30)	563 (31.90)	
Ilala	3 (27.30)	82 (29.50)	530 (30.10)	
Outside Dar	-	8 (2.90)	46 (2.60)	8.37 (0.76)
Parental Origin				
Coastal	3 (27.30)	102 (36.70)	505(28.60)	
Lake	1 (9.10)	28 (10.10)	*122*(6.90)	
Central	2(18.20)	61 (21.90)	*350*(19.90)	
Northern	1 (9.10)	19 (6.80)	288(16.30)	
Southern	3 (27.30)	44 (15.80)	362(20.50)	
Western	-	11 (4.00)	53(3.00)	
Zanzibar	1 (9.10)	13 (4.70)	83(4.70)	44.69 (0.01)

Hb FAD which is not part of SCD is excluded in this table, hence N = **2052*

***HbFA (n=1,678) included Barts (n=85) which are not considered as SCD*

9.7 Presence of Hb Barts and associated factors

A total of 109 Hb Barts were detected during HPLC sample analysis. Since it is well known that the level of Hb Barts varies depending on whether it is alpha plus, or alpha plus homozygous,[9] the Barts identified in this study were probably the homozygous alpha plus or alpha thalassaemia trait, and the ones with the not so obvious bands were just one chain deletions, alpha plus. Since in the Bio-Rad program we run, Hb Barts came off the column before integration begins, it was only seen by careful examination of the trace.

This finding necessitated a need for further analysis to establish the demographic and haematological factors that were associated with the presence of Barts. *Table 5* summarizes the findings obtained from the association between the presence of Barts and the associated factors. The results show that the association between age, sex gestation age and haemoglobin level were not statistically significant with the presence of Hb Barts ($p > 0.05$). The probability of having Hb Barts was highest among 10.1% and 36.7% of participants whose parents originated from Lake Zone and Coastal Region respectively while 5.5 % of participants originating from the Northern Region had the lowest probability of Hb Barts by nearly 3 fold (X^2=11.9, p=0.03). Presence of Hb Barts increased by nearly six times the likelihood of MCV being less than 100 fl in 78.2% of participants compared to 14% without Barts (X^2=2.3, p=0.001); and increased by 1.3 the chances of low birth weight in 33.9% compared to 25.4% of those without Barts (X^2=7.1 p= 0.03).

A few participants (16.1%) with MCH level less than 27 pg had a statistically significant increased probability of having Hb Barts as compared to (0.7%) of those who had no barts (X^2=1.5, p=0.001).

Table 5: Factors associated with presence of Hb Barts **N = 2053**

	HB BARTS		X^2 (P-value)
Age (Days)	**Present n (%)** N=109	**Absent n (%)** N=1944	
01 – 07	101 (92.70)	1758 (90.40)	
08 – 14	5 (4.60)	99 (5.10)	
15 – 21	3 (2.80)	55 (2.80)	
22 – 28	-	32 (1.60)	1.91 (0.59)
Sex			
Males	56 (51.40)	1082 (55.70)	
Females	53 (48.60)	862 (44.30)	0.77 (0.38)
Birth Weight (kg)			
< 2.5	37 (33.90)	493 (25.40)	
2.5 – 3.5	63 (57.80)	1135 (58.40)	
>3.5	9 (8.30)	316 (16.30)	7.12 (0.03)
Gestation age(weeks)			
< 37	24 (22.00)	339 (17.40)	
37 – 40	85 (78.00)	1605 (82.00)	1.49 (0.22)
Parental Origin			
Coastal	40 (36.70)	570 (29.30)	
Lake	11 (10.10)	140 (7.20)	
Central	21 (19.30)	393 (20.20)	
Northern	6 (5.50)	302 (15.50)	
Southern	19 (17.40)	390 (20.10)	
Western	5 (4.60)	59 (3.00)	
Zanzibar	7 (6.40)	90 (4.60)	11.89 (0.03)
MCV (fl)*			
< 100	68 (78.20)	206 (14.00)	
100 – 125	18 (20.70)	1243 (84.30)	
>125	1 (1.10)	26 (1.80)	2.34 (0.001)
HB level (g/dl)*			
< 14	22 (25.30)	361 (24.50)	
14 – 22	65 (74.70)	1087 (73.70)	
> 22	-	27 (1.80)	1.63 (0.44)
MCH (pg)*			
< 27	14 (16.10)	11 (0.70)	
>27	73 (83.90)	1464 (99.30)	1.51 (0.001)

**N = 1562 samples were only sufficient for complete blood counts*

9.8 Description of the haematological parameters of study participants

Table 6 and table 7 describe and summarize the haematological parameters (CBC) of study participants. Parameters are provided in mean and standard deviation categorized into sex and age groups. Total mean and standard deviation for the whole study participants are also shown at the end of each table.

Table 6: Profile of RBC value by sex and age **N = 1562**

		RBC	HB	HCT	MCV	MCH	MCHC	RDW
(Mean ± SD):		(μL)	(g/dl)	(%)	(fl)	(pg)	((g/dl)	(%)
SEX	**Male**	4.63 ± 1.07	15.72 ± 3.58	49.05 ± 11.53	106.19 ± 11.63	34.48 ± 5.55	31.95 ± 2.20	14.81 ± 1.81
	Female	4.66 ± 1.14	15.86 ± 3.46	49.47 ± 11.14	107.28 ± 9.38	34.71 ± 5.48	32.09 ± 1.59	14.51 ± 1.64
AGE (Days)	**1-7**	4.69 ± 1.11	16.01 ± 3.51	49.96 ± 11.31	107.29 ± 10.75	34.79 ± 5.67	31.99 ± 2.04	14.63 ± 1.77
	8-14	4.40 ± 1.02	14.44 ± 3.00	45.16 ± 9.71	103.31 ± 7.88	33.14 ± 2.91	32.06 ± 0.86	15.11 ± 1.45
	15-21	4.18 ± 0.83	13.23 ± 2.85	40.95 ± 8.82	98.06 ± 9.44	31.74 ± 3.28	32.31 ± 0.85	15.14 ± 1.44
	22-28	3.19 ± 0.97	12.98 ± 3.30	39.76 ± 10.91	101.56 ± 7.63	33.75 ± 4.82	32.48 ± 0.88	14.99 ± 1.14
Total Mean		4.65 ± 1.10	15.79 ± 3.53	49.23 11.36	106.67 ± 10.71	34.58 ± 5.52	32.01 ± 1.96	14.68 ± 1.74

Table 7: Profile of WBC and platelet counts by sex and age N = 1562

		WBC	LYM	MON	NEU	EOS	BAS	PLT
(Mean ± SD)		(x 10^9/L)	%	%	%	%	%	(x 10^9/L)
SEX	Male	12.92	44.93	15.31	30.58	2.07	5.62	160.47
		± 8.02	± 13.94	± 6.99	± 12.78	±3.71	± 6.55	± 91.61
	Female	14.06	44.98	14.41	31.30	1.72	5.58	167.22
		± 8.37	± 14.09	± 6.56	± 13.03	± 2.61	± 6.44	± 94.69
AGE	1-7	13.73	44.51	14.85	31.44	1.87	5.84	156.48
(Days)		± 8.34	± 14.03	± 6.76	± 12.92	± 3.35	± 6.58	± 85.64
	8-14	10.23	47.22	18.10	25.57	2.40	4.49	220.48
		± 5.24	± 13.95	± 7.51	± 12.81	± 2.38	± 6.99	± 121.54
	15-21	11.29	50.48	16.71	26.95	2.03	2.87	230.50
		± 7.54	± 11.99	± 6.67	± 10.38	± 2.24	± 2.93	± 122.59
	22-28	11.17	50.22	15.29	25.35	2.85	2.51	221.60
		± 5.73	± 13.25	± 4.22	± 9.91	± 3.46	± 2.44	± 155.79
Total		13.42	44.95	15.09	30.89	1.92	5.61	163.45
Mean		± 8.19	± 14.01	± 6.81	± 12.89	± 3.28	± 6.50	± 93.02

10. DISCUSSION

Neonatal screening for abnormal haemoglobins at MNH revealed the presence of normal (FA) and different types of haemoglobin variants including Hb FAS (sickle cell trait), Hb Barts, FS (SCA or HbS Beta ^{0} -thalassaemia), FSA (Sickle cell carrier or HbS Beta $^{+}$ -thalassaemia) and HbFA-D variant. The combined frequency for these identified abnormal haemoglobins was noted to be 18.2% of the total sample initially analysed by HPLC technique. Although the second line (confirmatory) test - Hb electrophoresis for abnormal haemoglobins was not able to provide conclusive findings, yet results provided in this study show an evidence of abnormal haemoglobins in this setting which has high level as well as dispersed haemoglobin variants.

10.1 Types of abnormal haemoglobins

The different types of abnormal haemoglobins identified in this study are similar to other publications that reported the importance of screening neonates for commonest abnormal haemoglobins, types and their frequencies that in turn would allow for strategies to ensure preventive measures and effective treatments are available and accessible. [1-11] It was therefore not surprising to find from this study that the frequency of abnormal haemoglobins is 18.2% (in which FS was 5 to 6 per 1000 and FAS was 14.04%) as similar finding was reported in previous study to calculate the prevalence of people with SCA done on the same setting at MNH. That study revealed that prevalence of people with the carrier state (AS) ranged from 15 to 20 % while the birth incidence of children with sickle cell anaemia (SS) in Tanzania was estimated to be 6 to 7 per 1000 children.[48]

Despite different methods applied for that study[48] including use of indirect method (Hardy-Weinberg Equation versus use of HPLC in this study), different study populations used (children population with mean age 9.7 $\pm$ 8 years versus neonates with mean age 1.2$\pm$ 0.5 days), and duration of time to conduct the study (5 years prospective surveillance cohort study versus three months cross sectional screening study), the consistence of results found for both studies highly suggests the likelihood that sickle

(S) carrier gene at Muhimbili National Hospital ranges from 14 – 20% of the general population and SCA (SS) ranges from 5 to 7 per 1000 live births.

Worldwide, the level of abnormal haemoglobins regardless of methods used to collect and analyse blood samples is well documented to be high amongst Africans.[10,15,18,52] This study supports the existing literature that the frequency of occurrence of abnormal haemoglobins in sub-Saharan Africa is high (15 – 25%) as compared to America and Europe where rates are lower (less than 9%) and mainly amongst black population.[33-36,39,41,53] Similarly, the rates of SCD were reported relatively low in America (1:375) and UK (0.3:1000) as compared to Jamaica (5.6:1000), Uganda (6.4:1000) and highest among Nigerians (18/1000).[2,18,21, 54]

10.2 Screening tools and types of abnormal haemoglobins

The effectiveness of methods used (HPLC for samples analysis) in this study may be considered successful, similar to many other neonatal screening programmes that utilized the same techniques to achieve the same objectives of establishing the frequency and types of abnormal haemoglobins.[1,3,39,47,49,60-61] Uses of HPLC and IEF as either screening tool and/or confirmatory test for abnormal haemoglobins is highly recommended amongst neonates since Hb electrophoresis is reported to be poorly conclusive as it was found in the results of this study. For example HPLC analysis results in this study identified 11 positive results for FS while only 3 samples were identified on Hb electrophoresis when used as a second line test. This observation is congruent to existing literature that the resolution between HbS and HbF on Hb Electrophoresis will be poor particularly with individuals with high HbF levels like in neonates. Therefore, relying on Hb Electrophoresis results during neonatal screening for abnormal haemoglobins may not be conclusive as many would show high rates of false negative results as compared to HPLC and/or IEF.[9,15,60,61]

Based on the above facts regarding the ill-defined effectiveness of Hb electrophoresis in identifying abnormal haemoglobins amongst neonates, the results of frequency and

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Based on the above facts regarding the ill-defined effectiveness of Hb electrophoresis in identifying abnormal haemoglobins amongst neonates, the results of frequency and

types of abnormal haemoglobins in this study were categorised by relying on only HPLC analysis results. Although parents were informed to repeat the Hb electrophoresis test after six months to confirm results and minimize the risk of false positive findings, this observation has necessitated the importance of having both IEF and HPLC in this setting to facilitate neonatal screening for abnormal haemoglobins as it is recommended in other studies.[9,15, 60] Nevertheless, utilizing HPLC technique has added a new knowledge for different types of abnormal haemoglobins that are possibly available or completely absent from this setting. For example it was previously known that sickle cell disorder (HbAS and HbSS) exist in Tanzania but the finding of Hb Barts, FSA (Sickle cell carrier or HbS Beta $^{+}$-thalassaemia) and HbFA D variant were only identified by the use of HPLC which is able to accurately quantify the Hb level and that Hb electrophoresis alone would have not been able to provide such additional knowledge.

The presence of Hb Barts is highly suggestive of α-thalassaemia though a molecular confirmatory test is required. The literature suggests that α-thalassaemia is found widely throughout sub-Saharan Africa, Mediterranean regions, the Middle East, and South East Asia.[9,15] This study has hence confirmed a possibility of α-thalassaemia in Tanzania and thus in sub-Saharan Africa though a molecular confirmatory test is required. FSA (Sickle cell carrier or HbS Beta $^{+}$-thalassaemia) was also an unusual identified haemoglobin variant with typical characteristics of having high percentage of HbF, followed by HbS and small percentage of adult haemoglobin; unlike FAS (sickle cell trait) which is characterised by high percentage of HbF, followed by almost equal percentage of HbA and HbS. It was possible to identify the different percentage of haemoglobin by the use of HPLC while Hb Electrophoresis would have not been able to distinguish the two (FAS and FSA).

Identification of Hb FSA has increased a probability of having Beta^{+}-thalassaemia which is a haemoglobin variant mainly found in the Middle East, and South-East Asia, Mediterranean countries and Northern/West Africa.[1,3,9,15] Finding FSA in this setting may be explained by the presence of study participants whose parents were residents from Lake and Coastal Regions but may have originated from the above mentioned

areas. Since Beta^{+}-thalassaemia (Hb FSA) is not well established in sub-Saharan Africa,[9] this study may therefore add knowledge for its existence in Tanzania either amongst African descendents or population from the endemic areas currently residing in Tanzania. HbFA-D variant is another uncommon Abnormal haemoglobins which was also identified by the use of HPLC for sample analysis. Its presence in this study is highly suggestive of either it may be found amongst Tanzanian population (1 per 2000 population) or from the descendants of North-West India, Pakistan and Iran where its availability has been documented by various authors.[8, 9,15,64]

Heel prick and cord blood collections are the two commonest methods applied when conducting neonatal screening for abnormal haemoglobins. However since cord blood is only relevant to the newborns soon after delivery, the method may not be applicable after day one of life hence deprive an opportunity for older groups of infants to participate in a screening programme.[10] Successful experience (99.2%) in utilization of heel prick method for blood collection in this study has supported the findings of other studies,[1,8,10,39] that this technique is reliable for use during neonatal screening as only 3.4% (n=70) of the samples collected had small clots.

Since HPLC requires only 5 µL of whole blood and 1 ml of haemolysis reagent for analysis, 53 samples with small clots sent to laboratory were successfully analysed by HPLC while 17 (0.8%) samples were only discarded. This observation is not different from other studies which utilized heel prick techniques as an effective method of blood collection for abnormal haemoglobins screening since it minimizes risks of infection during blood collection and has also minimal maternal blood contamination that can reduce the specificity of the results.[1,5]

10.3 Abnormal haemoglobins and associated factors

With the use of statistical significance test at 95% confidence level, this study noted that having abnormal haemoglobins increased the probability of neonates being born at low gestational age and that there were increased chances of abnormal haemoglobins amongst neonates whose parents originated from Lake Zone and Coastal Regions while

being born to parents originating from Northern Regions of Tanzania was identified as a low probability for abnormal haemoglobins. Other factors of interest assessed including age, sex, birth weight and place of residence, did not show statistical association suggesting that abnormal haemoglobins may not be influenced by them. This finding resembles other publications that the occurrence of abnormal haemoglobins is unevenly distributed throughout the world hence there are areas known to have high frequencies of occurrence while others have low occurance.[1-13] Anecdotal observation from clinical settings at MNH, has noted that the distribution of abnormal haemoglobins is reported to be high along the Lake Zone, Coastal Region and low risk along the Northern Zone of Tanzania. This study has therefore been able to exemplify the previous anecdotal observations in this clinical setting. Furthermore, it suggests that if a national neonatal screening programme for abnormal haemoglobins was to be implemented, people from the Lake Zone and Coastal Region should be given priority.

Since abnormal haemoglobins are genetic disorders with characteristic derangement at globins chain, and are known to be inherited as autosomal recessive,[9,15] it is therefore unlikely to find sex, age, and birth weight to be associated with hemoglobin disorders as portrayed by the findings of this study. However the presence of Hb Barts is known to be associated with premature delivery of newborns as the presence of α_2-thalassaemia could decrease the capacity to extract oxygen from the maternal circulation and hence contribute to early delivery of newborns with α_2-thalassaemia carrier, and in severe cases may cause fetal loss due to intrauterine anaemia and oedema (Bart's hydrops fetalis).[9,14,15] The above reported literature is exemplified in this study by showing that presence of Hb Barts significantly increased the probability of low gestation age ,low MCH (less than 27) and low MCV (less than 100fl) while Hb level was not significantly found to be associated with presence of Barts. Since it is known that individuals with homozygous α-thalassaemia are unlikely to be born alive,[9,15] this finding suggests that participants who were found with Hb Barts are likely to be heterozygous α-thalassaemia trait (carrier).

Presence of low MCV and MCH can be explained by that α-thalassaemia trait is characterized by a mild hypochromic anaemia with red cell indices similar to those of

the β-thalassaemia trait (low MCV).[9,15] Severe forms of α-thalassaemia is due to defective hemoglobin production which is non-functional and a haemolytic component. Furthermore, the result also suggests that neonates born with low MCV, and low gestation age should raise the suspicion index for α-thalassaemia (trait) as described earlier.

10.4 Haematological parameters

Haematological parameters (CBC) for newborns participating in this study were not different from many other authors who reported and recommended the levels to be utilized in neonatal clinical settings. The parameters generally did not show fluctuation between male and female participants but a significant range of values differed from one age group to another for all hematological parameters as is normally anticipated. Differences of complete blood counts in relation to age are widely reported since as neonates grow, they produce more of HbA (which has low affinity for oxygen), and HbF (which has high affinity for oxygen) diminishes.[9,15] For example the mean Hb level during the first week (1-7 days) of life in this study was reported as 16.04 $\pm$ 3.5g/dl, as compared to 12.98 $\pm$ 3.3g/dl reported at fourth week of life (22-28 days).

10.5 Study limitations

The extent to which the findings of this study can be generalised is limited by a relatively small sample size as compared to many other studies of similar objective. A selection bias that focused only on hospital based screening, and use of only one track alkaline (cellulose acetate) Hb electrophoresis as acidic (citrate agar) type was not available at MNH, could also limit the generalization of study findings. The limited duration of time to conduct the study, and the financial constraints of funding, these two factors mean that the data collection is from a small cohort of neonates from a relatively small geographical area. If funding and time had been available it would have been desirable to collect data from all across Tanzania, and from a much greater and diverse population. There is also a lack of availability of confirmatory (2^{nd} line) diagnostic testing such as isoelectric focusing and DNA analysis. Lack of further molecular (confirmatory) tests in this setting also limits proper diagnosis of abnormal haemoglobins such as thalassaemia.

11. CONCLUSION

From the findings of this study it may be concluded that the frequency of abnormal haemoglobins amongst neonates at MNH is high 18.2% (n=374) and therefore abnormal haemoglobins should be considered as a major public health problem in Tanzania.

The types and frequencies of the abnormal haemoglobins found in this study indicate presence of different types of haemoglobin variants including FAS (sickle cell trait), FS (SCA or HbS Beta^0-thalassaemia), Hb Barts, FSA (Sickle cell carrier or HbS Beta$^+$-thalassaemia) and Hb FA-D variant among study participants. There is pre-existing evidence that SCD (FAS and FS) is found in this setting but the knowledge regarding existence of Hb Bart, FSA and Hb – D variant at MNH is first disclosed by the findings of this study hence opening a wider understanding of haematological disorders in Tanzania.

The use of HPLC to screen for abnormal haemoglobins amongst neonates may be considered as an appropriate screening tool given that in this study Hb electrophoresis could not identify the majority (8 out of 11) of abnormal haemoglobins (SS) hence could had provided false negatives.

The findings of this study also concluded that abnormal haemoglobins were more likely associated with neonates being born with low gestation age; low birth weight; low MCH and low MCV. Participants whose parents' origins were from Lake Zone and Coastal Region of Tanzania had higher probability of having abnormal haemoglobins while being born by parents from the Northern Region of Tanzania had low probability of having abnormal haemoglobins.

12. RECOMMENDATIONS

This study has given evidence that screening for abnormal haemoglobins in this setting is feasible amongst neonates whose level were also found to be high. Therefore, it is highly recommended that a neonatal screening programme should be implemented as widely as possible so as to capture all affected neonates and hence provide opportunities to plan for preventive measures and allow for early identifications and treatment of related complications.

With consideration to resources available and cost effectiveness of screening programme, there is a need to consider the use of other screening tools recommended by many studies worldwide (e.g. IEF, immunological techniques such as ELISA etc) since Hb electrophoresis is not recommended by the findings of this study to be applied for neonatal screening due to its low sensitivity and specificity (increased high risks of false negatives) which may be fatal.

The results of this screening study will need molecular confirmatory tests to elicit the exact type of abnormal haemoglobins identified. Due to this limitation encountered during data analysis, this study therefore recommends additional efforts to ensure that molecular tests (DNA analysis by PCR) to confirm positive results of any given neonatal screening programme are also available.

HPLC as a neonatal screening tool is highly recommended for use in Tanzania due to its high sensitivity and specificity to capture and quantify different types of abnormal haemoglobins respectively which cannot be detected by Hb electrophoresis currently used by consultant and referral hospitals in Tanzania.

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