CHAPTER 1

1.0 INTRODUCTION

The inherited haemoglobin disorders are the commonest monogenic diseases worldwide. It has been estimated that approximately 7% of the world population are carriers of such disorders, and that globally 300,000-400,000 babies with severe forms of these diseases are born each year. [1, 2]

Inherited haemoglobinopathies fall into two main groups; the structural haemoglobin variants and the thalasaemias, in which there is absence or decreased synthesis of the haemoglobin chains. [2]

Although over 700 structural haemoglobin variants have been identified, only three, haemoglobin S, (HbS), haemoglobin C (HbC), and haemoglobin E (HbE), reach high frequencies. Haemoglobin E, which is the most common haemoglobin variant globally, is innocuous in its heterozygous and homozygous forms. The homozygous state for sickle cell gene (HbSS), on the other hand, is severe and results in sickle cell anaemia, a condition characterised by polymer formation of the haemoglobin chains under hypoxic conditions. [1]

The sickle cell gene is distributed widely throughout sub-Saharan Africa, the Middle East and parts of the Indian subcontinent and the Caribbean, where the carrier

frequency ranges from 4% to 40%. Europe, America, and the western pacific have a carrier frequency ranging from 1.1% to 4%. [2]

With a population of 650 million, sub-Saharan Africa has about 30 million children born with severe haemoglobin disorders. In Zambia the sickle cell frequency ranges from 6% to 27%. [1]

Infections are the most common cause of mortality in children with sickle cell anaemia. The organisms encountered are generally not unusual pathogens, but the infections they cause are more frequent and severe. Characteristically, the infections of sickle cell anaemia children are fulminant, without focus, and often fatal and typically caused by encapsulated streptococcus pneumoniae and to a lesser extent Haemophilus influenzae. [3]

In the USA and Jamaica infection is the commonest cause of mortality among sickle cell anaemia patients between the ages of 1-3years. [4]

In Zambia, infections are the most common cause of death among sickle cell anaemia patients aged between one and five years. Although infections have been noted to be the commonest cause of mortality in sickle cell anaemia patients less than five years of age, the common bacterial causes of infection among these patients in Zambia have not been reported. [5]

Interventions that have been targeted at reducing the burden of infection, especially against *Streptococcus pneumoniae* infection, in the form of pneumococcal vaccine and penicillin prophylaxis, in America and Europe, have significantly reduced the morbidity and mortality in sickle cell anaemia patients. [6, 7]

CHAPTER 2

2.0 STATEMENT OF THE PROBLEM

Like other malaria endemic areas in the subtropics, sickle cell anaemia is a disorder of public health importance in Zambia, with an average sickle cell trait frequency of 17.5%. [8]

Infections are the most common cause of death among Zambian sickle cell anaemia children. A study by Athale et al of 1994 observed that infections accounted for 29.84% of mortality among sickle cell patients. [5]

Despite infections being a common cause of morbidity and mortality, the causative bacterial organisms have not been reported. As such sickle cell anaemia patients presenting with fever are usually treated blindly with a combination of benzyl penicillin and chloramphenicol as first line treatment. In case of non response to these drugs, cefotaxime is then used as a second line drug. This treatment policy may delay appropriate treatment of the patients in cases where the prevailing organisms are not targeted since they are unknown, and this may as well increase the cost of treatment of these patients. It is, therefore, important to identify the bacterial organisms prevalent in these patients, and their sensitivity pattern.

CHAPTER 3

3.0 STUDY JUSTIFICATION

Studies on bacterial infections in children with sickle cell in Zambia were last done in the early 1990s. [5] Since then no recent studies have been done on the subject, and therefore, there is currently no information on the prevailing pattern of bacteraemia among children with sickle cell anaemia.

Therefore, there is need to know the currently prevailing aetiological agents of bacteraemia, and the microbial sensitivity pattern, in Zambian children with sickle cell anaemia. This information will help in the formulation of an appropriate antibiotic policy, which in turn will assist not only in reducing morbidity and mortality among these children, but also avoiding poly-pharmacy, thereby reducing the cost of treatment.

CHAPTER 4

4.0 LITERATURE REVIEW

4.1 Historical background

In 1910, Dr James Herrick presented a case report to the Association of American Physicians and later published it in the November 1910 issue of the Archives of Internal Medicine, of a Negro first year dental student- Walter Clement Noel, who presented 6 years earlier with severe respiratory problem. [9]

Noel was first seen by Dr. Ernest E. Iron an intern under Herrick. After performing routine physical, blood and urine examinations he noticed that Noel's blood smear contained many pear shaped and elongated forms of red blood cells.

In 1922, other publications of similar case reports led to the disease being named 'sickle cell'. [9]

4.2 Pathogenesis

The Sickle haemoglobin has arisen as a spontaneous mutation, (adenine to thymidine), in the gene that codes for glutamic acid as the sixth amino acid of the haemoglobin β-chain. This mutation substitutes valine (Val) for glutamic acid (Glu), in the β haemoglobin chain. [10]

The substitution of valine for glutamic acid leads to alteration in the charge, resulting in hydrophobic interactions with another haemoglobin molecule, under hypoxic conditions, thereby triggering an aggregation into large polymers. The polymerization

of deoxygenated haemoglobin S results in a distortion of the shape of the red cell and a marked decrease in its deformability. These rigid cells are responsible for the vaso-occlusive phenomena that are the hallmark of the disease. [11, 12]

Although sickle cell anaemia is a disorder of haemoglobin, leading to polymer formation and red blood cell sickling, the single most common cause of death in children with sickle anaemia is not polymerization but infection. [13, 14, 15, 16]

Sickle cell disease is inherited as an autosomal recessive trait. Homozygotes (inheritance of two abnormal genes) synthesize little or no haemoglobin A, and the red cells contain about 90% to 100% haemoglobin S, whereas heterozygotes (inheritance of one abnormal gene) have red cells containing about 20% to 40% haemoglobin S. [17]

4.3 Clinical Manifestation

The clinical manifestations of sickle cell anaemia are broadly as a result of shortened red cell survival due to haemolysis, vaso-occlusion, and the susceptibility to infection. These manifestations are age related.

The manifestations due to shortened red cell survival include anaemia, jaundice, gallstones, cardiomegaly, leg ulcers, marrow hyperplasia, poor physical development and delayed maturation. Manifestations due vaso-occlusion include painful crisis of bone and muscles, acute chest syndrome, strokes, eye damage, priapism, functional hyposplenism and chronic organ damage. [17]

4.5 Susceptibility to infection

Bacterial infections are a major cause of morbidity and mortality in children with sickle cell anaemia. Bacterial infection account for about 30 to 40% of mortalities in patients with sickle cell anaemia. The peak incidence is in the first 1 to 3 years of life and is infrequent after 6 years of age. This susceptibility age grouping corresponds to the period when most children lack circulating antibodies against the polysaccharide capsular antigen of streptococcus pneumoniae and Haemophilus influenzae type b. [18]

This syndrome of severe bacterial infection, results from two major abnormalities; absence of circulating specific antibodies, and splenic dysfunction.

Most children have not yet developed specific humoral immunity, during the first few years of life. In the absence of a functional spleen, these patients are at high risk for severe bacterial infections.

Children with haemoglobin SS disease have functional hyposplenism after infancy due to micro infarction, as a result of intravascular red cell sickling and recurrent occlusion of the splenic microcirculation. [19] The loss of splenic function has been demonstrated by reduced or absent uptake of technetium 99 metastable (99mTc), increased levels of pocked red blood cells (RBCs) and an increase in Howell Jolly bodies. [20, 21] This functional hyposplenism correlates with the level of fetal haemoglobin. Fetal haemoglobin forms hybrid tetramers with sickle haemoglobin, thereby interfering with polymerization of haemoglobin S in the solution phase. [22, 23, 24]

When the level of fetal haemoglobin decreased below 15 to 25 % splenic dysfunction usually occurs. Younger patients, less than six months of age, have higher fetal haemoglobin levels, normal uptake of 99mTc and lower percentages of pocked RBCs (25). The resultant splenic hypo function leads to defective opsonisation and phagocytosis of bacterial, especially the previously un- encountered encapsulated bacteria. [26]

The observed pattern of splenic dysfunction in sickle cell anaemia is also consistent with epidemiological data of increased rates of severe sepsis and meningitis caused by the aggressive encapsulated organisms, streptococcus pneumoniae and haemophilus influenzae type b. [27]

The splenic auto-infarction leads to loss of recognition and processing of particulate antigens that the host has not previously encountered, particularly carbohydrate antigens of encapsulated bacteria, and hence defective opsonisation and phagocytosis by splenic macrophages. [19, 28]

In addition the activity of compliment system in sickle cell patients has been found to be defective due to a deficiency of factor B, an essential factor in the activation of C3 convertase, an essential opsonin in the alternate arm of the compliment system. Similarly, Dieye et al demonstrated that patients with homozygous SS had reduced levels of C3c and immunoglobulin G (IgG). [29]

Several organisms, including streptococcus pneumoniae, haemophilus influenzae and non-typhi salmonella species have been identified as important causative organisms of severe bacterial infection in sickle cell anaemia patients. [16, 30, 31]

In a study of admission to hospital of 171 children with sickle cell anaemia, in South London, reviewed over a 20 year period, showed that 887 admissions occurred in 797 patients- years, the commonest cause of admission was painful vaso-occlusive crises followed by pulmonary disease, infection, anaemic episodes. Pneumococcal meningitis and acute splenic sequestration resulted in the most severe illness. [32]

Streptococcus pneumoniae was found to be the commonest cause of sepsis in young children less than two years of age, in the cooperative study of sickle cell disease. Out of a cohort of 600 patients 3.3% died. The commonest cause of death was infection. Eight of these deaths were due to infection caused by streptococcus pneumoniae. Eighteen episodes of pneumococcal sepsis occurred before one year of life. [33]

Hongeng et al, in a study of sepsis in sickle cell disease noted similarly, that streptococcus pneumoniae is the most common invasive infection among patients with sickle cell disease. He further observed that the risk of recurrent episodes of sepsis and subsequent death in those patients who have had a previous septic event is much higher. And, therefore, recommended that patients with sickle cell disease who have had pneumococcal sepsis should continue penicillin prophylaxis indefinitely. [34]

In a study to characterise recurrent infections in homozygous sickle cell disease, 214 episodes of invasive bacterial infections in 176 Jamaican patients with homozygous

sickle cell disease were examined. Streptococcus pneumoniae occurred in 81 episodes, Salmonella species in 70, Haemophilus influenza type b in 30, Escherichia coli in 24 and Klebsiella species in nine. The cumulative incidence showed that S pneumoniae and H influenza occurred predominantly before five years and was uncommon thereafter. [4]

This high prevalence of Streptococcus pneumonia as a cause of septicaemia in sickle cell anaemia patients has led to effective interventions in the management of sickle cell disease, thereby reducing the frequency of invasive Pneumococcal infections. These interventions include the use of Pneumococcal vaccination and antibiotic prophylaxis in children less than five years of age with sickle cell disease. [6, 7]

Earlier studies conducted in the region of Congo and Nigeria also demonstrated the high frequency of Pneumococcal septicaemia in African children with sickle cell disease.

A retrospective study of 69 case reports of children with homozygous sickle cell anaemia hospitalized from 1964 through to 1985 at the Kinshasa University Paediatric Hospital highlighted this patients' high susceptibility to infection. Among causative organisms, the most prevalent were salmonellae (20 cases), Pneumococcal (15 cases), and Klebsiella (12 cases). [35]

More recent studies, however, in tropical Africa have shown a different pattern in the bacterial agents of sepsis in sickle cell disease patients.

In a study to describe the pattern of septicaemia among sickle cell anaemia patients in Ibadan Nigeria, 269 patients with fever greater than 38C had blood cultures examination performed. Of the 97 patients who had positive blood cultures, 57 (59%) of the isolates were gram negative with Klebsiella sp. being the predominant species. While 40 (41%) were gram positive pathogens with staphylococcus aureus being the predominant species isolated. 31 patients had positive malaria parasites. [36]

In another study to describe the pattern of acute illnesses in homozygous sickle cell disease patients who attended the emergency department of a Lagos hospital, infections were found in 82% of all patients. The most common infections were pneumonia (35%) and septicaemia (32%). Klebsiella species was the predominant bacteria isolated accounting for 38%, followed by E. coli (23%) and staphylococcal aureus (23%). Staphylococcal albus and pseudomonas species also accounted for 23% each. [37]

Brown et al, in a study on childhood urinary tract infections observed that of the 171 urine specimens collected from 171 sickle cell patients, 37 had significant bacteriuria. The isolates were Escherichia coli, Klebsiella species, Non-haemolytic Streptococcus, Salmonella, Proteus and pseudomonas species. [38]

In a recent study of blood cultures in 155 homozygous SS Ugandan children with a fever of 38^oC or more, it was noted that streptococcus pneumonia was identified as a cause of infection in only 6% of the febrile episodes. The most common bacteria isolated were staphylococcal aureus (60%), followed by Haemophilus influenza (19%). [39]

This apparent lack of susceptibility to streptococcus pneumoniae infection in African patients with sickle cell disease has raised questions of the possible explanation. There are suggestions that malaria, with its associated splenomegaly, may favour persistence of splenic function during the critical period of susceptibility similar to that described in Eastern Saudi Arabia, where high levels of fetal haemoglobin may have a similar beneficial effect. The widespread use of over the counter antibiotics, as well as death of affected children before receiving medical attention, has also been suggested as possible explanations. [40]

CHAPTER 5

5.1 STUDY AIM

The aim of this study is to describe the pattern of bacteraemia among children with sickle cell anaemia presenting with fever at the University Teaching Hospital

5.2 SPECIFIC OBJECTIVES

1. To determine the common bacterial causes of fever among children with sickle cell anaemia patients at the UTH

2. To determine the antibiotic sensitivity pattern among the bacterial causes of fever in sickle cell anaemia patients at the UTH

CHAPTER 6

6.0 STUDY METHODOLOGY

6.1 STUDY DESIGN

This was a descriptive cross sectional study involving sickle cell anaemia patients, aged fifteen years and below, admitted to the paediatric wing of the University Teaching Hospital.

6.2 STUDY SITE

The study was undertaken at The University Teaching Hospital, a third level referral centre in Zambia. The hospital has a well established sickle cell clinic, which was established in 1973 by Chifumbe Chintu [5]. The clinic is conducted once a week and aims to offer comprehensive health care to patients with sickle cell disease. The participants were recruited from the department of paediatrics and child health of the University Teaching Hospital. The recruitment point was the admission ward. Patients from the sickle cell clinic and the emergency room were both admitted to the admission ward.

6.3 STUDY DURATION

The study was carried out from 1st of April 2009 to 30th November 2009, covering a period of eight months.

6.4 STUDY POPULATION

The study population comprised of sickle cell anaemia patients aged fifteen years and below who presented with axillary temperatures of 38.0^{o}C and above, at the University Teaching Hospital.

6.5 SUBJECT SELECTION

The criteria for inclusion were:

1. Confirmed sickle cell anaemia patients, known or newly diagnosed by haemoglobin electrophoresis, with axillary temperatures of ≥38°C, aged fifteen years and below. The diagnosis of sickle cell anaemia was confirmed using the Shandon alkaline electrophoresis machine.

2. Consent to enrol into the study by parent or guardian. Assent was sought for patients aged eight to fifteen years.

The exclusion criteria were as follows:

1. Non confirmed cases by Hb electrophoresis
2. Patient age more than fifteen years
3. Axillary temperature of less than 38°C
4. Lack of consent, and assent where applicable

6.6 SAMPLE SIZE AND SAMPLING

The sample size was calculated using the following formula, adapted from WHO;

Sample size = 4 X proportion X (1-proportion) X design effect

Margin of error X margin of error

Proportion of sickle cell admissions = 3%

Design effect = 2

Margin of error = 0.05 (assumed to be)

Therefore, sample size = $\frac{4 X 0.03 (1-0.03)2}{0.05 X 0.05}$ = 93.12

Expected drop out rate = 20%

Final sample size = 120 participants.

All the sickle cell anaemia patients admitted to the paediatric wing of the University Teaching Hospital during the study period who met the inclusion criteria were enrolled.

A convenient sampling method was applied.

6.7 DATA COLLECTION

1. A structured questionnaire was used to collect socio-demographic data (i.e age, sex and residential area), frequency of admissions to hospital in the past one year, use of antibiotics prior to presentation and the frequency of routine sickle cell clinic attendance. A sample of the questionnaire is attached in the appendices.

2. A data capture sheet was used to record the results of the Laboratory and radiological results (i.e. FBC, ESR, blood culture, urinalysis and urine culture, malaria parasite slide, and chest X-ray). A sample of the data capture sheet is as shown in the appendices.

6.8 STUDY PROCEDURE

The study was conducted with the help of two research assistants, hemato-oncology unit doctors and nurses both in the outpatient and the hemato-oncology ward. The research assistants were involved in explaining the purpose of the study and obtaining consent from parents and guardians. Both the principal investigator and the research assistants were involved in the collection of specimens, while transportation of the specimens to the laboratory was done by the research assistants.

The parents and guardians were interviewed so as to gather and document the socio-demographic parameters, frequency of disease events, and use of antibiotics as well as attendance to the sickle cell clinic.

Patients were evaluated by the attending physician, and the author was responsible for the investigations.

On enrolment the following investigations were performed:

1. Complete blood count; blood was drawn from each participants and transported to the laboratory in an EDTA specimen bottle within thirty minutes to one hour of collection, for determination of the complete blood

count. The blood count was performed using Sysmex Xs 800i an automated hematology analyzer, which performs analysis of WBC and differential with an optical detector block based on the flow cytometry method using a semiconductor laser. The RBC and platelet count are analysed by the RBC detector using the hydro dynamic focusing method.

2. Blood culture: every participant had blood drawn from them under aseptic conditions, which included swabbing the selected areas with iodine and methylated spirit. The preferred site of blood collection was the cubital fossa, and a minimum of 2.5mL of blood was collected for blood culture testing from each participant. The blood specimen was then transported to the laboratory in a commercially prepared blood culture media specimen bottle (BD BACTEC Plus Aerobic/F and Plus Anaerobic/F media) within one hour of collection.

3. Urine: a urine sample was collected from every participant by clean catch method for microscopic examination and culture. The samples were transported to the laboratory in plain vacutainer specimen bottles immediately after collection. A separate sample was collected for urinalysis.

4. A blood smear was also collected from every patient for malaria examination in the laboratory.

5. HIV serology was routinely done on all patient admitted to the admission ward after counselling by trained counsellors who were stationed right in the ward, in accordance with the policy of counselling and testing every patient admitted to the hospital regardless of their condition. The HIV serology was performed using Determine HIV-1/2, an

immunochromatographic test for the qualitative detection of antibodies to HIV 1 and 2, as a screening test and the Trinity Biotect Uni-Gold as a confirmatory test. Only those who could not give consent did not have the test done.

6. Other investigations such as radiological examination, cerebral spinal fluid examination and indeed any other tests were performed at the discretion of the attending physician.

6.9 LABORATORY PROCEDURES

Urine Sample

A clean catch mid stream urine sample was collected in a sterile container from all the participants. A clinitest was performed on the freshly voided urine sample, by the bed side, and the urine leukocytes and nitrites were recorded onto the data capture sheet. Another urine sample was transported to the laboratory in a sterile container within one hour of collection. The samples were then examined macroscopically for the colour, turbidity and odour.

After macroscopic examination, a well mixed sample of urine was then inoculated onto blood agar and MacConkey agar using a sterile standardised loop. The plates were then incubated aerobically at $37^{o}C$ for 18 - 24hrs.

The remaining sample of urine was centrifuged at 2500-3000rpm for 5 minutes. The supernatant was then decanted and the urine deposits examined microscopically using the x10 and x40 objectives.

On day two, the plates were examined for pure growth and a colony count was performed on positive pure growths. Appropriate identification tests as well as susceptibility tests using recommended antimicrobial agents were carried out.

During the period of the study susceptibility testing was available for the following antibiotics; Penicillin, Cefotaxime, Nalidixic acid, Norfloxacin, and Ciprofloxacin

Blood samples

Venous blood was collected into a blood culture specimen bottle (BD BACTEC Plus Aerobic/F and Plus Anaerobic/F) and transported to the laboratory immediately after collection. The site of veno- puncture was swabbed with iodine before being dried with methylated spirit to ensure aseptic blood collection. Adult specimen bottles were used during the study because the microbiology laboratory does not procure paediatric blood culture bottles.

The blood culture bottles were then incubated at $37^{o}C$ overnight, in a BACTEC 9050 BD. (The available BACTEC machine has a capacity of only 50 specimen bottles for incubation at any one time. Therefore any extra specimen bottles would have to wait until the next cycle).

On day two, the bottles were gently removed from the incubator, and examined macroscopically for turbidity, haemolysis, gas production and bacterial colonies. A sub culturing was then done. One mL of broth culture was withdrawn using a needle and syringe after disinfecting the cap with 70% alcohol. The MacConkey agar plate was then incubated aerobically at $37^{o}C$ for 18 - 24hrs, while the blood agar plate and chocolate agar at $37^{o}C$ in 5% CO_2 for 18 - 24hrs. The blood culture bottles were then re-incubated.

On day three, the sub cultured plates were read and identification, and susceptibility testing done on pure growths. Culture plates with no growth were re – incubated under conditions described above.

On day four, all plates re-incubated on the previous day are read, and identification and susceptibility testing done on pure growths. Plates with no growth were again re-incubated

The same processes of re-incubation and sub-culturing were repeated on days 5, 6 and 7. Appropriate tests were performed on plates with pure growth on day 8 for identification and susceptibility testing, and those with no growth were discarded.

6.10 Ethical approval

The study was approved by the Biomedical Research and Ethics committee of the University of Zambia.

Consent was granted by the guardians and or parents of the patients prior to enrolment in the study. Assent was sought for patients aged above eight years. (a copy of the consent form is as attached in the appendices)

CHAPTER 7

7.0 RESULTS

The study criteria were fulfilled in fifty five participants during the study period. All the 55 participants had both blood and urine samples drawn from them and were available for analysis. Eleven participants had chest X-rays done on them on clinical suspicion of pneumonia.

7.1 SOCIO-DEMOGRAPHIC CHARACTERISTICS

Figure 1; Age Distribution
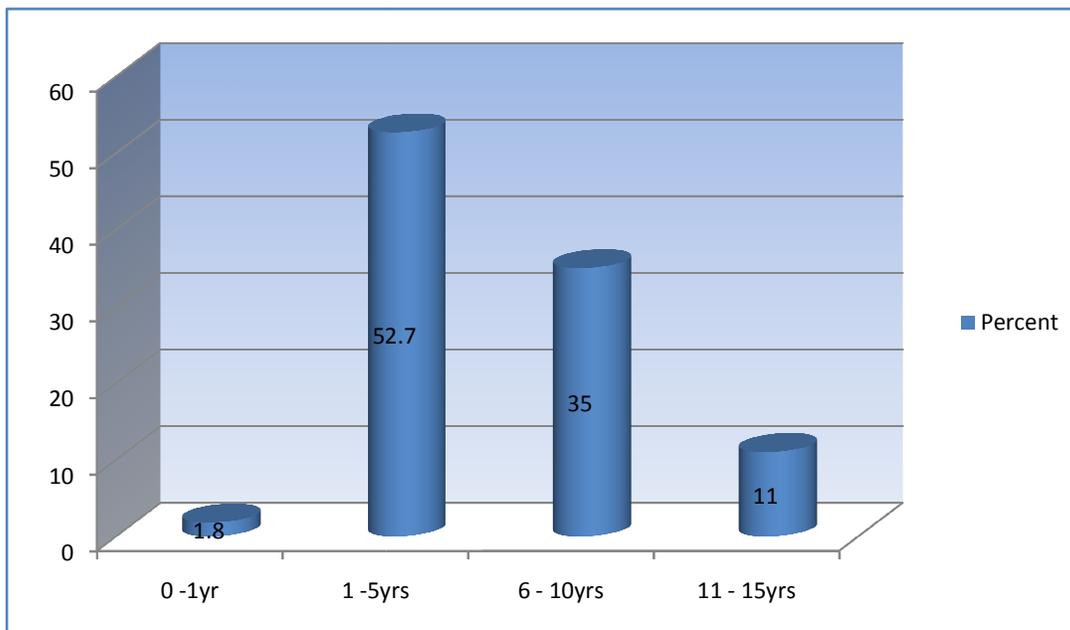
Over 50% (29) of the sickle cell anaemia patients, who presented with fever during the time of the study, were aged less than five years. The median age of the participants was 7.5 years (range 11mo - 14yrs).

55 %(30) of the participants were male, with a male to female ratio of 1.2:1.

The majority of the participants (31) came from the densely populated townships of Lusaka (i.e. Matero, Chawama, Kalingalinga, and Mtendere)

7.2 CLINICAL CHARACTERISTICS

Figure 2; Age at diagnosis
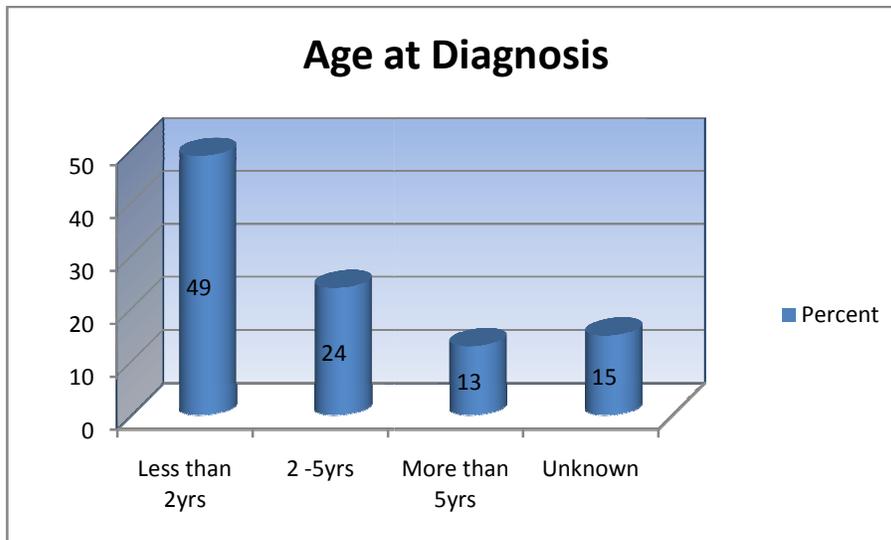
About half (27 out of 55) of the enrolled patients had the diagnosis of sickle cell anaemia made when they were less than two years of age. However, thirteen percent (7) of the participants had their diagnosis made when they were more than five years of age.

Table 1; Common symptoms associated with fever

Symptom	Number	Percent (%)
Body pains	24	43.6
Cough	21	38.2
Diarrhoea	4	7.3
Vomiting	3	5.5
Difficulty in breathing	3	5.5
Dysuria	1	1.8
Others	6	10.9

Body pain was the most common associated symptom accounting for 38.9% of symptoms presented followed by a complaint of cough. Symptoms included in the 'others' category are sore throat, poor appetite, heart palpitation and dizziness.

Thirty eight (21) percent of the participants admitted to having used at least one antimicrobial in the last seven days prior to presentation to hospital. Of those who had used antibiotics at all, the majority had taken them for 3-5 days.

Seventeen of the twenty one had only used one antimicrobial, whereas two of them used two antibiotics and one participant had used a combination of three different antibiotics.

The commonest antibiotic used was amoxicillin accounting for 60% (12) of the respondents.

Figure 3; Common antibiotics used prior to admission
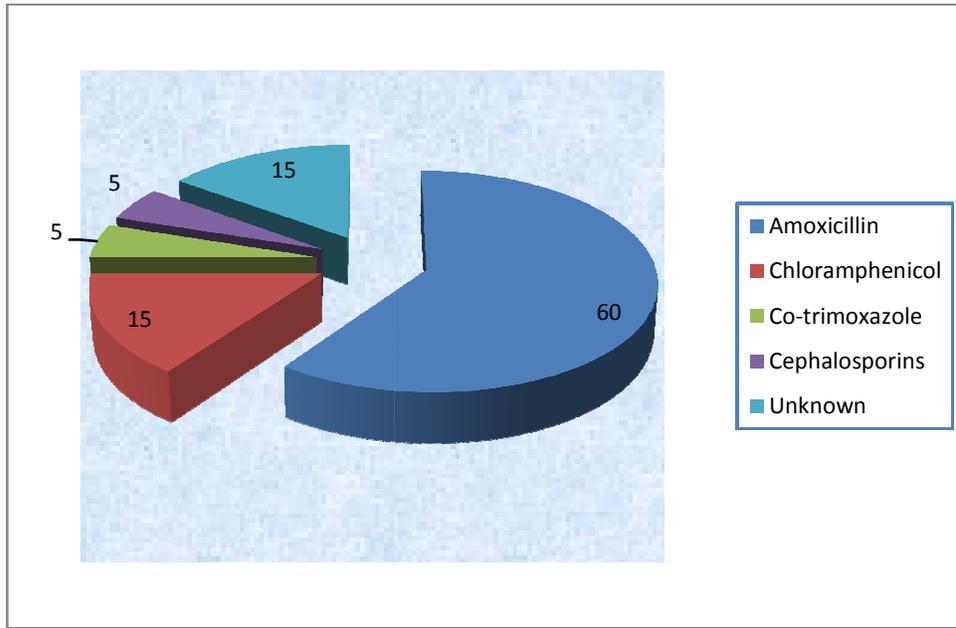
About 80% (43) of the participants attended the sickle cell clinic consistently in the past one year (i.e. had missed not more than one review in the last one year).

About 75 %(41) of the respondents reported having been on regular malaria prophylaxis, with deltaprim (a combination of dapsone and pyrimethamine), as per sickle cell clinic protocol. Most of the patients who were not on malaria prophylaxis cited difficulties in accessing the drug as the main reason.

Almost all the enrolled patients were on folate supplementation accounting for about 95% (52) of the participants.

Of the enrolled patients, 51 of them took the HIV test, and none of them was found positive. The HIV counselling and testing was done as a routine procedure for all the patients admitted to the hospital

7.3 LABORATORY RESULTS

Figure 4; WBC count levels
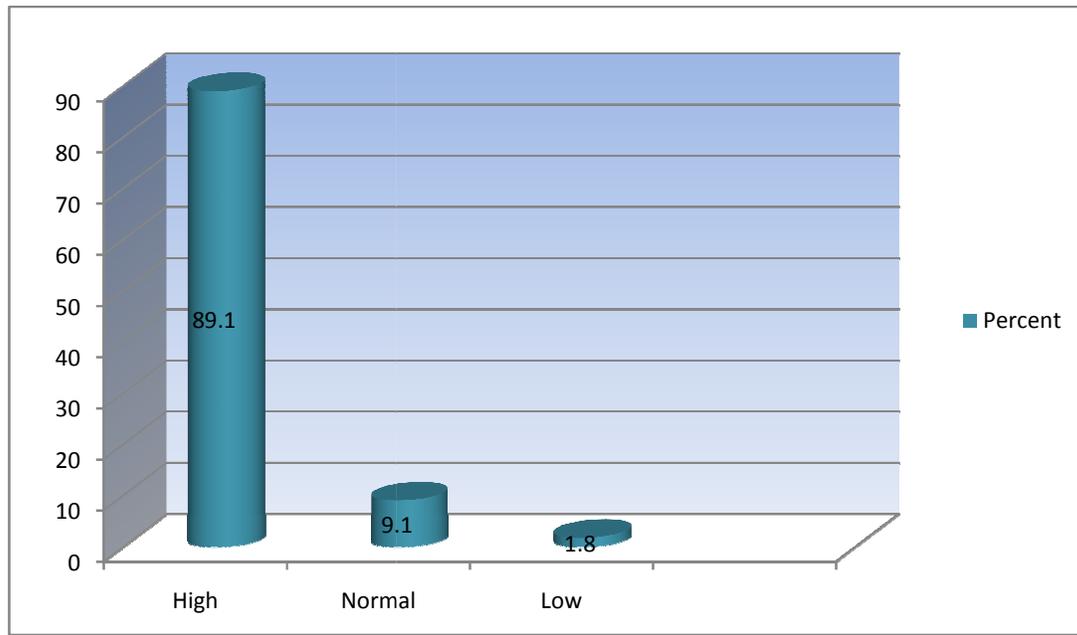
Note;

    a. High WBC count refers to values above 12×10^9

    b. Normal WBC count refers to values between 4 and 12×10^9

    c. Low WBC count levels refers to values below 4×10^9

    d. The WBC values were not corrected for nucleated RBCs

The majority (89%) of the patients had a high WBC (White Blood Cell) count with values exceeding 12×10^9. 1.8% of the patients had low WBC count with values less than 4×10^9.

Figure 5; Urinalysis results
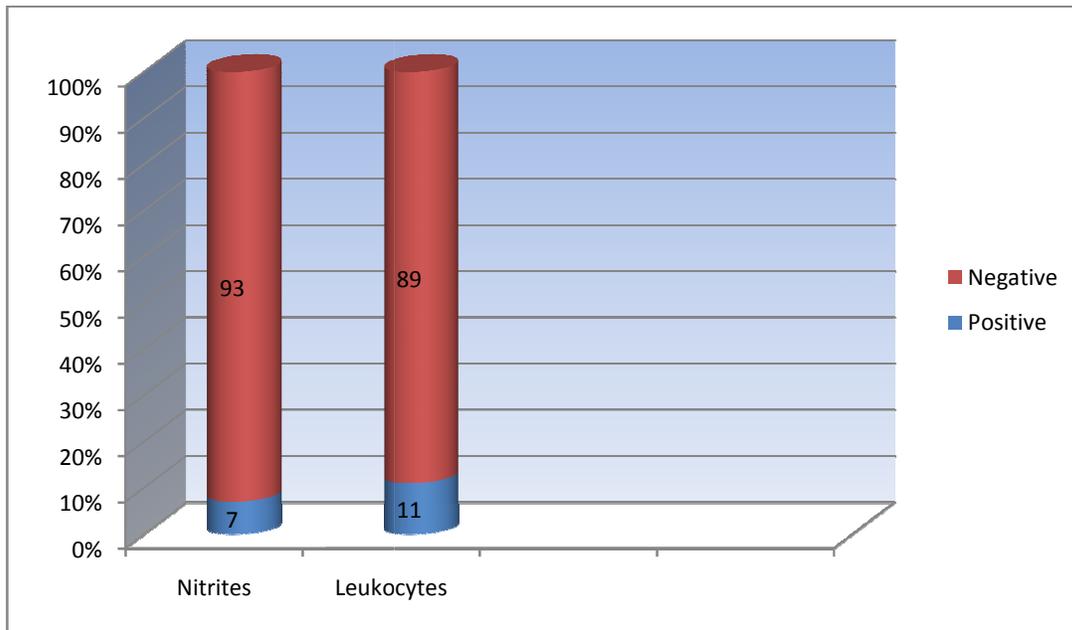
Four (7.3%) of the urine samples collected were positive for nitrites. Leukocytes were positive on six (10.9%) samples. Four out of the fifty five participants had positive bacterial urine cultures accounting for over seven percent of the participants. The isolated organisms and their associated characteristics are as shown in the table 2 below.

Table 2; Urine culture bacterial isolates and associated characteristics

Bacteria Isolates	Escherichia coli	Citrobacter diversus	Enterobacter aerogens	Streptococcus species
Age(yrs)	5	6	10	12
Sex	female	male	male	male
Fever	38.6^{o}C	38.7^{o}C	38.0^{o}C	38.0^{o}C
Urine nitrites	positive	negative	positive	positive
Urine microscopy (leukocytes)	numerous	20	numerous	10

Note; numerous refers to more than 40 pus cells per high power field

Table 3; Urine microscopy results

	Nil	<5	5-10	10-40	>40
Pus cells	37	12	3	1	2
Red blood cells	55	0	0	0	0

The table above shows urine microscopy results. 6 out of 55 patients had more than 5 pus cells per high power field. All the samples examined were negative for blood.

Malaria parasite smears were done on all the enrolled participants to exclude plasmodia infection as a common cause of fever in these patients. All the smears were reported negative for malaria. Antigen or antibody based malaria tests were not performed due to limited resources.

Table 4; Blood culture bacterial isolate and associated characteristics

Bacterial isolate	Streptococcus species
Age	1year 4 months
Sex	Female
Residence	High density area (Matero)
Associated symptoms	Fever, cough and diarrhoea
Degree of fever	38.0^{o}C
Use of antibiotics prior to presentation	None
Susceptibility results	Penicillin, Cefotaxime, Norfloxacin

Only one of the blood samples collected was reported positive on culture accounting for 1.8%. The blood culture isolate was identified as Streptococcus species sensitive to Penicillin, Cefotaxime and Norfloxacin

The only positive blood culture sample was from a 1yr 4mo old female toddler from a high density residential area of Matero.

Eleven out of fifty-five (20%) participants had chest X-rays done as was clinically indicated. Out of the eleven, seven films were reported as abnormal and had opacities suggestive of consolidation.

CHAPTER 8

8.0 DISCUSSION

This study was conducted from April 2009 to November 2009 covering a period of eight months. During the period of the study a total of 199 sickle cell patients were admitted to the admission ward of the paediatric wing of the UTH. Fifty five patients out of the 199 met the criteria for inclusion into the study, accounting for 27.6% of the total sickle cell related admissions. This figure falls short of the targeted sample size due to two main reasons. The first reason is the fact that during the time of the study health workers at the UTH were on industrial strike for about two months. During this period the institution was only attending to emergencies, as a result the number of patients presenting to the hospital drastically reduced. In the same vein the number of sickle cell patients presenting to the UTH markedly reduced, thereby affecting the number of patients recruited into the study. The second reason is that very few of the patients being treated clinically as sickle cell patients had their diagnosis confirmed by Hb electrophoresis. As a result they did not meet the inclusion criteria and were excluded from the study.

8.1 Socio – demographic factors

The age of the enrolled patients ranged from seven months to fourteen years, with a mean age of 7.5years. Participants aged between one and five years accounted for 52.7% of the enrolled patients. This finding is similar to that of Barclay et al who noted that mostly the diagnosis of sickle cell anaemia is made after one year of age in Zambia. [40]

However, of the new patients enrolled at the sickle cell clinic between 2004 and 2009, the majority of the paediatric patients were aged between 5 and 15years indicating that fever was more common in the younger age group, i.e. less than five years of age.

The male to female ratio among the study participants was 1.2: 1, similar to the findings by Chintu et al who reported, in a study of 1994, that of the sickle cell patient mortalities 37 were male while 25 were female, giving a male to female ratio of 1.5:1. [5]

Most of the enrolled patient came from crowded peri - urban areas of the city, accounting for about 56.4%. Participants from low density areas contributed 1.8% of the study population. Similarly the majority, about 62%, of the new patients enrolled at the sickle cell clinic for about six years (2004 – 2010), were from the high density peri-urban areas of Lusaka.

8.2 Clinical characteristics

Of the enrolled participants 49% of them had the diagnosis of sickle cell made at less than two years of age. A significant proportion had delayed diagnosis. And about 13% of them were diagnosed at more than five years of age. The reasons for the delay in diagnosing sickle cell anaemia are beyond the scope of this study. However it will be reasonable to assume that among other factors, lack of laboratory facilities for the diagnosis of sickle cell anaemia is contributory. This factor is suggested by the finding that the majority of the patients admitted to the admission ward with sickle cell anaemia, during the duration of the study, had the diagnosis of sickle cell anaemia made clinically and sickling test alone.

Hb electrophoresis was not routinely done because the electrophoresis machine being used in the haematology laboratory is obsolete and frequently broke down.

All the enrolled patients had significant fever as per enrolment criteria, 78% of them had fever ranging from 38.0^{o}C to 38.9^{o}C. Patients with fever of less than 38°C were excluded from the study because they were less likely to have significant bacterial infection, however there is no absolute set of numbers that separate febrile children into bacteraemic or non bacteraemic groups. [41, 42]

8.3 Laboratory and Radiology results

Urine results

Four out of the 55 urine samples, yielded significant bacteriuria of ≥ 100, 000 CFU/mL accounting for 7.3%. Samples that did not yield significant bacteriuria were not reported as positive yields. The organisms isolated include *Escherichia coli*, *nonhemolytic streptococcus species, citrobacter diversus* and *enterobacter aerogens*. The patients with positive urine cultures were all aged five years and above.

This finding is consistent with the findings in other parts of Africa that reported urinary tract infections to be more common in older children with sickle cell anaemia. [31, 38] One out of the four participants was female. All of the participants were from high density residential areas, and one of the four positive cultures had a history of use of antibiotics prior to presentation.

Malaria parasite smear

None of the parasite smears done yielded a positive result. This finding differs from that by Chintu et al of 1994, in which malaria contributed about 9% as a cause of death in sickle cell patients

This difference could be due to the fact that the study by Chintu et al focused on the mortalities, whereas this study focused on sickle cell patients who presented with fever. The high levels of malaria prophylaxis in the sickle cell clinic which is at 75%, as well as the general decline in the national malaria prevalence which is currently at 10.9%, and Lusaka in particular at 1.7%, according to the Zambia demographic and health survey of 2008, are probably the major contributors to the zero prevalence of malaria parasites on smears. In addition, the departmental laboratory records revealed that during the duration of this study, the malaria parasite positive smear results stood at 0.8%, that is 7 positive smears out of 847 slides examined. The blood smears for malaria, however, were not accompanied by antigen based rapid diagnostic testing due to limitation in resources.

Radiology

Seven out of the eleven chest radiographs done on clinical suspicion of pneumonia, revealed opacities in the lung fields, suggestive of either pneumonia or micro-infarcts in the lung fields. Three of the seven radiographs with opacities had associated granulocyte predominant leucocytosis. This is strongly suggestive of bacterial infection. This group of patients could have contributed to the fever with blood culture negative results as bacterial yields on blood cultures are generally quite low in pneumonia.

Blood specimen

Of the total specimens, one yielded positive isolates on blood cultures accounting for 1.8%. The isolate was identified as a non haemolytic streptococcus species. This isolation rate is lower than reported in other literature. Kizito et al reported 28% bacterial isolation despite the 25% reported use of antibiotics use prior to admission to hospital. The common organisms isolated include *staphlycoccus aureus*, 28%, *haemophilus influenzae*, 9%, and *streptococcus pneumoniae* accounted for only 3%. [39] Whereas Williams et al reported 6% bacteraemia, i.e. 108 episodes out of 1749 sickle cell anaemia admissions, over a period of ten years in the Kalifi district of Kenya. The commonest bacterial isolate was found to be *streptococcus pneumonia* 25.9%, and *staphylococcus aureus*, 10.5%, among the gram positives as well as *non- typhi salmonella*, 11.7%, and *haemophilus influenza* at 6.1% of the isolates among the gram negatives. [43] Lobel et al reported 10.5% bacteraemia in sickle cell anaemia children, 22 out of 210 patients.

This poor bacterial yield of the blood specimen samples could be due to high levels of use of antibiotics, 38%, especially amoxicillin which accounted for 60% of the antibiotics used. However, as noted above, Kitizo et al reported a much higher microbial yield despite the use of predominantly penicillin prior to admission, in their study population. The other contributing factor could be due to the fact that only one blood specimen was collected per participant instead of the recommended three samples due to limited supply of specimen bottles. This assumption could not be compared with similar studies because there was no clear mention of the number of blood specimens collected per participant.

The use of adult specimen bottles, which require considerably more blood (10mL of blood per sample), could also have affected the bacterial yield. The other contributing factor is the limited capacity of the available BACTEC machine, which could have lead to considerable delays in processing the samples with consequent low culture yields. A much higher bacterial yield was expected, especially that the majority of the participants enrolled in this study were aged between 1 and 5 years, a period of maximum susceptibility to infection among sickle cell anaemia patients.

Most of the patients had associated granulocytosis (54%), which is suggestive of possible bacterial infection. However, as pointed out above, the bacterial yield from blood cultures did not demonstrate bacterial infection. In addition the presence of fever in sickle cell anaemia could be due to the release of pyrogens during the process of red cell sickling and occlusion of the microvasculature. [10]

CHAPTER 9

CONCLUSION

Bacterial infections are a common cause of fever in sickle cell anaemia patients leading to significant morbidity and mortality. Five of the fifty five participants had confirmed bacterial infection on culture. This accounts for about 10% of the study population. Urinary tract infection contributed about 7.3%, whereas 1.8% had bacteraemia. Another 5.5% of the participants had clinical, radiological, and laboratory evidence strongly suggestive of bacterial infection.

Blood culture positivity rate in this study was lower than the findings of similar studies in the region. The low bacterial yield on blood cultures in this study could be due to the use of adult blood culture bottles, the relatively small number of study participants and the prior use of antibiotics by some participants.

Due to the low bacterial yield on bacterial cultures, the findings of this study do not conclusively show the common causes of bacteraemia in sickle cell anaemia patients with fever. This is mainly due to the relatively small number of patients enrolled.

CHAPTER 10

10.0 RECOMMENDATIONS

1. A similar study needs to be carried out with particular attention to the following points;

    a. A bigger sample size of about 120 participants or more, as calculated above should be attained.
    b. A fully functional Hb electrophoresis machine should be in place for confirmation of the diagnosis of sickle cell disease.
    c. Paediatric blood culture bottles should be used for blood specimen collection instead of the adult type.
    d. Molecular techniques such as PCR may be used to increase the isolation of bacteria from the specimens.
    e. A BACTEC machine with a larger capacity should be used to avoid delays in processing the specimens

CHAPTER 11

11.0 LIMITATIONS

1. Lack of a fully functional Hb electrophoresis machine at the institution, during the duration of the study, for confirmation of the diagnosis, leading to a number of potential candidates being excluded from the study.

2. Lack of appropriate paediatric specimen bottle affected the quality of specimens delivered to the laboratory.

3. Limited capacity of the BACTEC machine leading to delays in incubating the samples may have contributed to the low bacterial yield on cultures.

4. Health workers' industrial strike at the UTH during the period of the study contributed to fewer number of patients being enrolled into the study

CHAPTER 12

12.0 REFERENCES

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CHAPTER 13

13.0 APPENDICES

13.1 Enrolment form

1. Participants details---------------------

a. Date------------------------

b. Study ID number-----------------------

c. Hospital number------------------------

d. Age (yrs) -------------------------------- Date of birth-----------------------

e. Sex------------------------------

f. Residential address--------------------

g. When was diagnosis made------------------ How was it diagnosed-----------

clinically-------------- sickling test------------Hb electrophoresis------

2. Presenting complaints.

    a. fever---------------------

    b. others--------------------

    c. number of hospital admissions in the last one year---------------------

    d. number of febrile illness in the last one year------------------------------

    e. when was the last admission---

3. Use of antibiotics

a. use of antibiotics in the last one week. Yes ------------. No-------------------

b. Type of antibiotics used

    i. Amoxicillin-----------------------------------

    ii. Co-trimoxazole-----------------------------

    iii. Erythromycin------------------------------

    iv. Chloramphenicol--------------------------

    v. Cephalosporins (specify) -----------------

    vi. Others (specify) ----------------------------

c. Dosage ------------------- ---------------------- --------------------

d. Duration ------------------ ---------------------- -------------------

4. Sickle cell clinic attendance in the last one year

a. Yes----------------- No-----------------------------

b. How often---

c. Malaria prophylaxis. Yes------------------- No-------------------

d. Folate supplementation. Yes ----------------------. No---------

e. HIV status. Positive ---------------. Negative--------------------

13.2 Data Capture Sheet

A. Study ID number -------------------------

B. Investigations done

     i. Chest X-ray

     ii. Full blood count

         WBC---------------- Granulocytes--------------------

         RBC---------------- Lymphocytes-------------------

         Hb------------------ Monocytes-----------------------

         MCV----------------- Eosinophils----------------------

         PLT------------------

     iii. Erythrocyte sedimentation rate (ESR) --------------------

     iv. Blood culture result---------------------

     v. Urinalysis result. Leukocytes ---------. Nitrites---------Specific Gravity (SG)----

     vi. Urine culture results-----------------

     vii. Malaria parasite slide. Positive --------------. Negative----------------------

     viii. Cerebral spinal fluid. Organisms--------------------------

         Biochemistry. Protein-----------. Glucose------------. Chloride. ----------

13.3 Information Sheet

Introduction

My name is Dr Mbinga LM, I am a post graduate student at the University of Zambia School of Medicine, Department of Paediatrics and Child Health. I am conducting a study in sickle cell children that present with fever at our hospital. The purpose of this study is to try and identify the common micro-organisms that are causing fever in these patients*.*

Invitation

You are, therefore, invited to participate in this study that is looking at the common bacterial causes of fever in Zambian sickle cell anaemia children. The study is being conducted in order to identify and possibly prevent or minimize these infections in sickle cell patients.

Nature and purpose of the study

The study is being conducted in view of the fact that sickle cell patients are susceptible to infections. Knowledge of the common infecting organisms may help health care providers to prevent these infections and/or better treat your child and many others with sickle cell anaemia.

Procedures of the study

If you agree to participate in the study, we will obtain information from you regarding age of the child, admissions and social data. Samples of the urine, blood and any other tests relevant to the illness of your child will be drawn and transported to the laboratory for testing. The results of the tests will be communicated to you if you so

wish.

Possible risks and discomforts

Your child will not be exposed to any risk by enrolling into the study. The child will however experience discomfort from routine collection of blood samples and any other procedures during the course of treatment of your child as part of his/her routine hospital care.

Possible benefits

Apart from receiving treatment for his or her current infection, the recommendations generated from this study will contribute to the body of knowledge and help improve care of your child and many others.

Confidentiality

All the information collected in this study is strictly confidential. Data that will be collected and reported will not include your name or your child's name and will not appear on the study files and therefore cannot be traced to you.

Your participation in this study is strictly voluntary. You and your child will not suffer any consequences if you decide not to participate in the study. You may also withdraw from the study at any time for any reason without consequences to you or your child's care.

13.4 CONSENT FORM

I, _____ hereby confirm that I have been sufficiently explained to about the nature, conduct benefits and risks of this clinical study. I have also received, and/or read and understood the above written information about the study. I am aware that my personal details and that of my child will be anonymously processed into the research report. I have understood that I may voluntarily at any point, withdraw my participation and that of my child from the study without suffering any consequences. I have been given sufficient time to ask questions and seek clarifications, and of my own free will declare my participation and that of my child into the research study.

I have received a signed copy of this agreement.

Participants' signature or thumb print _____ date ____/_____/_____

Person obtaining informed consent _____ date ____/_____/_____

Thank you for considering you and your child's participation into the study. If you have any questions, concerns and clarifications, please contact Dr Mbinga or the University of Zambia, Biomedical Research Ethics Committee on the following addresses;

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