PREVALENCE OF IRON DEFICIENCY ANEMIA IN CHILDREN WITH MALARIA

BY

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DECLARATION

I hereby declare that this dissertation represents my own work and has not been presented either wholly or in part for a degree at the University of Zambia or any other University.

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APPROVAL

This dissertation of Dr Evans Mulendele is approved as partial fulfillment of requirements for the award of Master of Medicine in Pediatrics and Child Health by the University of Zambia.

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ABSTRACT

Background

Both iron deficiency and malaria on one hand are common in sub-Saharan Africa and the interaction between the two is complex. On the other hand both cause anemia which is one of the most prevalent diseases suffered by individuals in developing world. The etiology of anemia is frequently multi-factorial, with iron deficiency, malaria, hemoglobinopathies and other nutritional deficiencies contributing to its cause. This study assessed the prevalence of iron deficiency during the acute episode of malaria illness and during convalescence.

Methods

This was a prospective case study and a clinical assessment was used to collect information at the time of enrollment (day 0) and convalescence (day 35). In addition laboratory assessment was conducted on all the recruited children on day0 and day 35 which included full blood count and sickling test. Hematological indices of interest in this study were hemoglobin (Hb), mean Corpuscular Volume (MCV), mean cell hemoglobin (MCH) and red cell distribution width (RDW). The Hb, MCV, MCH and RDW were used to determine iron deficiency. The children who had MCV less than 70 fl, MCH less 27 (pg) and RDW percentage more than 16 % were said to have iron deficiency and those who in addition had HB < 12g/dl were defined as iron deficiency anemia. The children with positive malaria parasite slide (ring forms of plasmodium falciparum) were defined as having malaria.

Results

Prevalence of iron deficiency was thirty-nine percent (39%) and those with anemia were thirty five percent (35%) at presentation (day 0). The number of children who were iron deficient on day 35 increased to fourty nine percent (49%). Fifty percent (50%) of children with severe anemia (Hb < 6g/dl) had iron deficiency at day 0. Children who had anemia with no iron deficiency at day 0 were 34%, however on day 35 the 18% of the children with anemia and no iron deficiency developed iron deficiency. The changes in iron status between days 0 and day 35 were statistically not significant in all the parameters used to determine iron deficiency. But individually there were some children whose anemia status worsened on day 35.

Conclusion

This study has shown that iron deficiency (shown by the hematological indices of MCV less than 70 fl, MCH less 27 (pg) and RDW percentage more than 16 %) is prevalent among children with malaria and in some cases iron deficiency worsens during convalescence. How much iron deficiency impacts on malaria anemia cannot be concluded from this study. More studies need to be done especially to asses the benefits of supplementation with iron in a child after an episode of malaria. Such studies should involve both rural and urban populations.

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LIST OF ABBREVIATIONS

ID	Iron deficiency
IDA	Iron deficiency anemia
WHO	World Health Organization
ZP	Zink Protoporphyrin
SF	Serum Ferritin
TfR	Serum Transferrin Receptors
UTH	University Teaching Hospital
Hb	Hemoglobin
HCT	Hematocrit
RBC	Red Blood Cells
PTL	Platelets
MCV	Mean Corpuscular Volume
MCH	Mean Cell Hemoglobin
MCHC	Mean Cell Hemoglobin concentration
RDW	Red Cell Distribution Width
MAC	Mid Arm Circumference

CHAPTER ONE

1.0 INTRODUCTION

Anemia remains one of the most prevalent diseases suffered by individuals in developing world and is a critical co-morbid factor contributing to the excess morbidity in these regions. (WHO 2001). The etiology of anemia is frequently multi-factorial, with the relative contribution of iron deficiency, malaria, hemoglobinopathies and other nutritional deficiencies. The prevalence of iron deficiency, which is usually detected by low serum ferritin concentrations, is estimated to be 2.0 to 2.5 times the prevalence of anemia. There is little data on the prevalence of iron deficiency in developing countries because of limitation in resources required to measure the necessary biochemical indicators. Therefore, anemia prevalence can generally be taken as an indicator of the extent and trends of iron deficiency. [1] However in many developing countries, anemia can also result from infections such as malaria, from chronic inflammatory disorders or from other nutritional deficiencies of folate or vitamin B-12. Serum ferritin levels are measured in patients as part of the iron studies workup for anemia. The ferritin levels measured have a direct correlation with the total amount of iron stored in the body. If ferritin is high there is iron in excess, which would be excreted in the stool. If ferritin is low there is a risk of lack in iron which sooner or later could lead to anemia. In the setting of anemia, serum ferritin is the most sensitive laboratory test for iron deficiency anemia. [2] As ferritin is also an acute-phase reactant, it is often elevated during the course of any acute infection. A normal C-reactive protein can be used to exclude elevated ferritin caused by acute phase reactions. In addition it is well known that

infection and inflammation influence hemoglobin and iron status indices such as zinc protoporphyrin (ZP) and serum ferritin (SF) [3] and, therefore, obscure the detection of iron deficiency. Accurate estimates of the prevalence of iron deficiency and IDA are not available in many non-industrialized countries. In this study mean corpuscular volume (MCV), mean corpuscular hemoglobin or mean cell hemoglobin (MCH), mean corpuscular hemoglobin concentration or mean cell hemoglobin concentration (MCHC) and Red cell distribution width (RDW) were used to determine the iron deficiency. Anemia caused by iron deficiency is called microcytic hypochromic anemia which is characterized by small and paler red blood cells than usual on microscopic examination. The normal mean corpuscular volume is 76-100 fl, with smaller cells (<76 fl) described as microcytic and larger cells (>100 fl) as macrocytic. The paleness of the red blood cells can be quantified as the mean corpuscular hemoglobin or mean cell hemoglobin (MCH), the amount of hemoglobin per cell; being less than the range of 27-32 picograms (pg). Iron deficiency anemia is by far the most common cause of microcytic anemia. The other less common causes are thalassemia; sideroblastic anemia, congenital or acquired; sometimes, anemia of chronic disease, although this more typically causes normochromic, normocytic anemia and rarely lead poisoning. The red blood cell distribution width is a measure of the variation of red blood cell width that is reported as part of a standard complete blood count. Usually red blood cells are a standard size of about 6-8μm. Certain disorders, however, cause a significant variation in cell size. Higher RDW values indicate greater variation in size. Normal range of variation in human red blood cells is 11 - 15%. RDW test results are often used together with MCV results to figure out what the cause of the anemia might be. It is mainly used to

differentiate between iron deficiency anemia, in which RDW is elevated, and other microcytic anemias. [4]

In population groups with a high prevalence of anemia, however, almost all individuals will be iron deficient, except possibly if the anemia is caused by malaria only. That iron deficiency adversely affects human health is widely recognized. Iron plays a critical role in the transport of oxygen throughout the body and in cellular processes of growth and division. Iron deficiency results in a decrease in the hemoglobin concentration, which when sufficiently low is identified as anemia.

Malaria is caused by protozoan parasites of the genus Plasmodium. Four species of Plasmodium can produce the disease in its various forms: Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale and Plasmodium malariae. P. falciparum is the most widespread and dangerous of the four and if untreated it can lead to complicated malaria and even death.

In contrast, malaria causes anemia through cytokine-mediated suppression of hematopoeisis, and in addition, when infected with P. falciparum, the erythrocyte changes make it vulnerable to early clearance. Both iron deficiency and malaria are common in much of sub-Saharan Africa, and the interaction between these conditions is complex. The question is whether children with iron deficiency are prone to develop severe malarial anemia or have protection from severe anemia. Hookworm and other infections that cause blood loss also contribute to iron deficiency and anemia.

All types of anemia, regardless of cause, reduce the oxygen transported throughout the body, and this leads to decreased productivity and increased risk of cardiovascular events. Other poor health outcomes associated with iron deficiency and anemia include

poor neurologic development in children, premature labor, low birth weight, and increased maternal and infant mortality. [5,6,7]

Iron deficiency is the most common and widespread nutritional disorder in the world [8]. As well as affecting a large number of children and women in non-industrialized countries, it is the only nutrient deficiency which is also significantly prevalent in virtually all industrialized nations. There are no current global figures for iron deficiency, but using anemia as an indirect indicator it can be estimated that most preschool children and pregnant women in non-industrialized countries, and at least 30-40% in industrialized countries, are iron deficient [9,10]. A similar proportion of anemia was found to be alleviated with iron supplementation in clinical trials from malaria-endemic regions, indicating similar beneficial hematological effects from iron supplementation in both malaria endemic and non-malarious regions. This does not mean, however, that malaria is not a fundamental cause of the severe anemia observed in children in malaria-endemic areas. For example, a clinical trial in Tanzania compared malaria chemoprophylaxis with iron supplementation in infants, and demonstrated that both iron supplements and chemoprophylaxis reduced severe anemia, but malaria chemoprophylaxis reduced severe anemia by 60% compared with 30% reduction in those receiving iron supplements [2,11]. Despite the efficacy of supplementary iron for the prevention and treatment of iron deficiency and anemia, debates over the use of iron supplements in malaria-endemic regions continue because of concerns that they may increase susceptibility to malaria. [12,13]

Therefore iron deficiency is responsible for the majority of anemia worldwide, but in malaria-endemic regions, malaria may be responsible for the majority of child mortality

due to severe anemia. How much iron deficiency contributes to an increase in mortality secondary to anemia in these malaria-endemic areas is not known. Hence the importance of determining the prevalence of iron deficiency in children presenting with malaria. This will help in decision making and recommendations to introduce iron supplementation in malaria control programs.

1.1 STATEMENT OF THE PROBLEM

The prevalence of iron deficiency in developing countries is high. Zambia is a malaria endemic area and anemia is one of the major complications of this parasitic disease. [14] How much iron deficiency contributes to an increase in mortality secondary to anemia in these malaria-endemic areas is not known.

Hence the importance of determining the prevalence of iron deficiency in children presenting with malaria. This study wanted to establish what proportion of children with malarial also have iron deficiency. The study also looked at the iron status of children with malaria during convalescence.

1.2 MAIN OBJECTIVE.

To determine the iron status of children during malaria episode and convalescence

1.2.1 SPECIFIC OBJECTIVES.

1. To find out the prevalence of microcytic hypochromic anemia in children with malaria episode and during convalescence.
2. To determine the Red Cell Distribution width (RDW), mean Cell Volume (MCV) and Mean Cell hemoglobin (MCH) as additional way of assessing iron deficiency in the study population.

CHAPTER TWO

2.0 LITERATURE REVIEW.

Sources of iron include liver and red meat like beef, beans and peas. The form of iron found in meats is called heme iron, and is much more bioavailable (absorbable) than the type of iron (non-heme) found in plants. Non-heme iron, found in beans, peas, and non-animal sources, which accounts for more than 85% of iron in the average diet, is not absorbed as well as heme iron. Absorption of non-heme iron is increased when consumed with animal protein and vitamin C.

Iron deficiency can be considered as a continuum from iron deficiency with anemia to iron deficiency without anemia, to normal iron status with varying amounts of stored iron, and to iron overload – which causes organ damage when severe. Nutritional iron deficiency is a common problem in poor families where the children do not consume much in the way of animal products. Other conditions such as malaria, helminths and chronic disease can also play a role in the causal path. Iron deficiency is the result of long-term negative iron balance leading to depletion of hemoglobin. This results from diminished supply of iron to the transport protein apotransferritin which in turn leads to decreased transferrin saturation and an increase in transferrin receptors in circulation and on the surface of cells, including erythrocytes.

The early stages of iron deficiency can be recognized by abnormalities in serum ferritin (SF), zinc protoporphyrin (ZP), and serum trasferrin receptor (TfR), whereas the more advanced stage of iron deficiency, iron deficiency anemia, occurs when anemia develops.[15,16,17,]. At University Teaching Hospital, 10 to 15 % of children with

presumptive diagnosis of malaria have severe anemia (Hospital records – HMIS 2005). Anemia is the major cause of mortality in children with malaria in the first 48 hours of admission at University Teaching Hospital (Hospital records – HMIS 2005)

Literature regarding the relationship between malaria and iron deficiency has been dominated by studies in which micronutrient has been given therapeutically, either as prophylaxis or for treatment of the deficiency. Although early studies of this kind have suggested that iron supplementation might be associated with an increased incidence of clinical malaria [18, 19]. This suggestion has not been confirmed in studies conducted more recently [20]. Nevertheless, a recent meta-analysis has shown that the evidence still favors a small but significantly increased risk of malaria after iron supplementation. [21]. Relatively little is known about the converse association- that is the incidence of malaria among iron deficient children. It is not well established whether iron deficiency has adverse or protective effects against infectious diseases.

The nutritional immunity theory was developed from studies that observed a protective effect of iron deficiency on malaria severity. [22]. The theory suggests that depriving the parasite of essential nutrients (iron, in particular) creates an uninhabitable internal environment, thus preventing the parasite from fully proliferating. In 1985, a study in mice found that the entire iron-replete group of mice infected with P. chabaudi died, whereas the iron-deficient mice were far less likely to die. When the iron-deficient mice were then fed iron-sufficient foods, they fell victim to recrudescent parasitemia. [23,24]. More recent experimental evidence refutes the earlier nutritional immunity hypothesis and concludes no significant protection of iron deficiency in young rats. [25]. Other evidence suggests that iron deficiency impairs immune responses, including

T lymphocyte production and activity, natural killer cell activity, and neutrophil function. [26].

A meta-analysis of twelve published and unpublished placebo-controlled iron supplementation trials to examine the effect of iron supplementation on malaria morbidity showed that there was a significantly heightened risk of infection as measured at the end of the study associated with malaria and iron supplementation. Iron supplementation appeared to increase other malariometric indices, including risk of a malaria attack and spleen enlargement although the increases were not significant. Thus, iron supplementation had the unintended consequence of marginally increasing certain malariometric indices in clinical trials. The hematological improvements in the iron supplementation groups summarized over the studies were significant and included an average increase in hemoglobin levels of 1.2 g/dl and a 50% decrease in the risk of severe anemia. [27]. Consensus has not yet been reached on the risks and benefits associated with iron supplementation and malaria morbidity and mortality. Although more research is indicated, current knowledge suggests that the alleviation of anemia through iron supplementation is likely to benefit all iron-deficient populations, including those in malaria-endemic regions.

As already noted both iron deficiency and malaria are common in much of sub-Saharan Africa, and the interaction between these conditions is complex. To investigate the association between nutritional iron status, immunoglobulins, and clinical Plasmodium falciparum malaria, a study was done to determine the incidence of malaria in a cohort of children between the ages of 8 months and 8 years who were living on the Kenyan coast. Biochemical iron status and malaria-specific immune responses were determined

during 2 cross-sectional surveys. It was found that the incidence of clinical malaria was significantly lower among iron-deficient children. [28]. But this information has not been reproduced anywhere else. It is also not known whether iron deficiency protects against severe forms of malaria especially anemia.

In African children a question may be asked whether anemia is commonly secondary to malaria or iron deficiency especially in malaria endemic areas. In Kenyan children there is a report that iron supplementation had a greater effect on the hemoglobin concentration than intermittent administration {every 4 weeks} of sulfadoxine-pyrimethamine. These findings and those from other randomized placebo-controlled trials provide some answers to the question raised above, while taking into account the degree of endemicity and distinguishing between effects on mild to moderate and on severe anemia [29]. Another aspect of the interaction between iron deficiency and malaria needs to be considered. What is the effect of malaria on iron deficiency? Bearing in mind that iron deficiency and Plasmodium falciparum malaria are the two main causes of anemia in young children in region endemic for this disease. The impact on iron status of prophylactic oral iron supplementation (2 mg/kg/day from two to six months of age) and the duration of this effect were assessed in a group of 832 Tanzanian infants exposed to P. falciparum malaria. Iron parameters and red blood cell indices were assessed at 2, 5, 8, and 12 months of age. Infants who received iron supplements had a significantly lower prevalence of iron deficiency. Red blood cell indices (mean corpuscular volume, mean cell hemoglobin, and mean cell hemoglobin concentration) were increased in children receiving iron supplementation and they did not differ between those protected and unprotected against malaria. The prevalence of

ferropenia was similar in children protected against malaria and in those who were not protected and did not receive iron supplements. It was concluded that iron supplementation between the ages of 2-6 months improves iron status at least up to 12 months of age. Malaria infection does not contribute to iron deficiency [30].

In the last two decades, health authorities and policy makers have increasingly recognized the importance of iron deficiency and anemia as a public heath problem. This is reflected in the goals on the reduction of iron deficiency anemia endorsed by heads of states, ministers in the world declaration and plan of action from the world summit for children (1990) and in the world Declaration plan of Action for Nutrition from the International Conference on Nutrition. As noted before that consensus has not yet been reached on the risks and benefits associated with iron supplementation and malaria morbidity and mortality [30], there is need to determine concurrent prevalence of iron deficiency in children with malaria.

CHAPTER THREE

3.0 METHODOLOGY

3.1 STUDY DESIGN.

The study was a prospective case study of iron deficiency in children admitted to University Teaching Hospital with malaria positive blood slide. The study was nested within an ongoing study evaluating the effect of monocyte inhibiting factor (MIF) on the etiopathogenesis of severe malaria anemia.

3.2 STUDY POPULATION.

3.2.1 Site Description.

Children were recruited from the outpatient department, admission ward and pediatric wards at the University Teaching Hospital, Zambia. The University Teaching Hospital is a tertiary hospital, which receives referral cases from all levels of health care in Lusaka and the whole of Zambia. Malaria transmission in this area occurs throughout the year, although the majority of clinically evident infections occur after the period of rainfall that generally occurs during the months of October to March. Due to low incidence of malaria cases at the initial chosen site, three more sites were included in the study namely Chongwe clinic, Chipata Clinic and Mpongwe Mission Hospital. UTH, Chonngwe clinic and Chipata clinic are in the Lusaka Province of Zambia, while Mpongwe Mission Hospital is on the Copperbelt Province of Zambia. UTH and Chipata clinic cater more of urban population where as Chongwe Clinic and Mpongwe Mission Hospital which cater more a rural population.

3.2.2 Subject Selection.

Screening criteria

Any Child between the age of 2 months and 5 years with axillary temperature of 37.5o C or more and clinically suspected of having malaria.

Recruitment inclusion criteria

1. Any child admitted or seen at UTH Department of Pediatrics and Child health, Chipata clinic, Chongwe clinic and Mpongwe Mission Hospital with a provisional diagnosis of malaria who had blood drawn for malaria parasite and hemoglobin.
2. Confirmed positive malaria parasite slide.(Ring forms of plasmodium falciparum)
3. Those with haemoglobin < 6g/dl were recruited in the severe malaria group and those with haemoglobin > 6g/dl were recruited in the uncomplicated malaria group

Recruitment exclusion criteria

1. Sickle cell disease and any other known chronic illness or immune deficient disease including HIV infection.

Sampling guide for patients recruited and sample handling.

Children with hemoglobin less than 6g/dl were recruited as severe malaria anemia where as those with hemoglobin more than 6g/dl as uncomplicated malaria. The hemoglobin indices of these children were checked at the time of admission and during convalescence on day 35. In this study, it would have been better to get a third arm of

patients with no malaria as control, but due to logistical problems it was not possible. It was going to be difficult to get blood from health controls. Nevertheless the information, which was gotten from the study, was enough to answer the study question. The study looked at the hemoglobin indices of children with uncomplicated anemia and children with severe anemia on day 0 and day 35. The blood was drawn by the researcher and the samples were analyzed at the University Teaching Hospital Pediatric Laboratory and Mpongwe Mission Hospital laboratory.

3.2.3 Sampling frame.

Time frame: The study was done for 12 months

Sample size:

The sample size of 150 in this study was calculated at the confidence interval of 95% with a P-value of 0.05 using the formula by Whitley E. and Ball J. [31]

$$N = \frac{2}{SDD} \times Cp \quad \text{and} \quad SDD = \frac{\text{target difference}}{SD}$$

Where n = sample size, SDD = Standardized difference

In this case SDD was worked out by taking the reported 40% Iron deficiency prevalence (WHO) under five years .The study hypothesis was that children with severe malaria anemia will have a higher iron deficiency prevalence of 60% hence

$$SDD = \frac{\text{Target effect}}{SD} = \frac{0.6-0.4}{0.3} = \frac{0.2}{0.3} = 0.67.$$

95% confidence level at P-value of 0.05 is given as 13 and therefore the estimated sample size was 38 and with a drop out rate of 25% the estimated sample size for this

study was 50 giving a total sample size of 100 for the two study groups. The incidence of malaria was very low and some of the patients would not consent to participate in the study hence only managed to follow up 38 patients which is 50% of the sample size.

3.2.4 Subject management.

Diagnosis of Iron Deficiency

Examination of the full blood count and blood film will usually suggest the diagnosis. It should be emphasized that not all hypochromic/microcytic anemias are due to iron deficiency. The Red Cell Distribution Width (RDW) is a useful parameter in differentiating iron deficiency from thalassaemia minor by providing a measure of variation in red cell size. In thalassaemia minor, the RDW is usually normal as the population of cells are relatively uniform, whereas in iron deficiency, the value is usually >14.5%. The gold standard for the assessment of stored iron is marrow aspiration and Prussian blue staining. Alternatively, a therapeutic trial of oral iron will confirm the diagnosis if a reticulocytosis (at one week) and a rise in hemoglobin (usually in the order of 0.5 to 1.0 g/week) are found. The iron deficiency was estimated using MCV, MCH, MCHC and RDW. Hence the conclusion of the study may be affected by the above problem but as mentioned the above parameters (MCV, MCH, MCHC and RDW) have been proven to be good indicators of iron deficiency and are preferred in acute inflammatory disease states. [32,33]

The researcher and research assistant administered a questionnaire to every recruited child's parents or guardian.Patients were seen on the day of recruitment to the study (day 0) and 35 days later during follow up (day 35). For every study patient, 3 mls

venipuncture blood was collected at day 0 and day 35 for research purposes. The full blood count was done using an ABX MICROS 60 (automated machine. Thick malaria parasite slides were taken from the study patients for microscopic studies after staining with Giemsa stain. Patients were on antimalarial treatment according to the national treatment guidelines and the clinical judgment of the attending clinician. Those children with severe malaria received quinine, while those with uncomplicated malaria were given Artemether-Lumefantrine (Coartem).

3.2.5 Data management.

The Epi info statistical software was used to compute the iron status in children with acute malaria and during convalescence. The results were also tabulated and compared between day o and day 35.

3.2.6 Ethical issues.

This study was carefully explained to every accompanying responsible adult of every child recruited in the study. Both written and verbal consent (or and ascent) was obtained (see appendix 4). The study was approved by the research and ethics committee of the University of Zambia. For the additional sites permission was obtained from the research ethics committee of the University of Zambia and the Ministry of Health.

CHAPTER FOUR

4.0 RESULTS

A total of thirty-eight children were recruited in the study. The sample size could not be reached due to the low incidence of malaria which was over estimated in the calculation of the sample size. This is due to the fact that malaria incidence has drastically gone down. The age range of these children is shown in table 4.1. The majority of these children were between the age of 12 and 36 months. 19 were male and 19 female as shown in table 4.2. At enrollment 84 % of the children were well nourished, 8% at -1 Standard Deviation and 8% at -2 Standard Deviation (table 4.3) and there was no change on day 35 (data not shown). Table 4.4 shows the means, Standard Deviation and P- Values for day 0 and day 35, respectively of Hb, HCT, MCV, MCH, MCHC and RDW. Six (15.7%) children had severe anemia (Hb < 6 g/dl) , moderate anemia (Hb 6≤10 g/dl) 8 (21 %), mild anemia (Hb 10≤12 g/dl) 12 (31.5%) and those with no anemia (Hb > 12 g/dl) were 12 (31.5 %) Table 4.6 shows the iron status of the children using the variables which are used to define iron deficiency in this study. The prevalence of iron deficiency was thirty-nine percent of the children in the study. Most of the children presented with a fever of less than one week duration. Convulsions were only present in two children prior to admission. Three children presented with signs of heart failure and were given blood transfusion. The other prominent symptoms were diarrhea and vomiting.

Table 4.1: Age range of enrolled children in months

2-12months	12-24months	24-36months	36-50months
8	10	14	6
21 %	26 %	37 %	16 %

Figure 4.1, Age range.

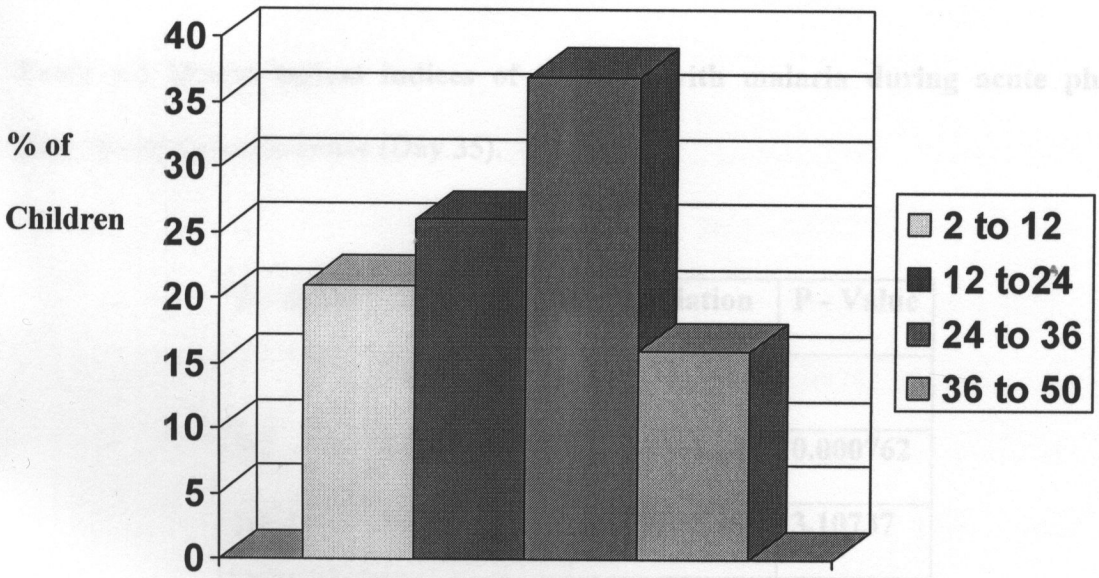

Table 4.2: Sex distribution

Sex	Female	Male	Total
Number of children	19	19	38
Percentages	50%	50%	100%

Table 4.3: Nutritional Status of the children at recruitment

Z – Scores	Median	- 1 SD	- 2 SD	Total
Number of children	22	2	2	26
Percentage	84%	8%	8%	100%

Table 4.4 Hematological indices of children with malaria during acute phase (Day 0) and convalescence (Day 35).

Variable	Mean±Standard Deviation		P - Value
	Day 0	Day 35	
Hb	10.005±2.887	12.015±1.848	0.000762
HCT	26.395±7.971	33.545±5.239	3.10737
RBC	3.874±1.174	6.6481±0.553	0.000628
MCV	70.108±7.877	71.719±8.584	0.422466
MCH	26.342±4.674	26.645±4.714	0.542061
MCHC	37.034±5.610	36.581±4.081	0.703546
RDW	15.868±2.165	16.510±1.892	0.18058

Table 4.5: Mean and Standard Deviation of severe, moderate, mild anemia and normal hemoglobin on day 0 and 35

HB status		< 6g/dl	6 to 10 g/dl	10 to 12 g/dl	> 12 g/dl
Hb	O	4.9+_1.0844	8.3+-1.3259	10.9+-0.418	12.8+-0.6379
	35	10.9+-1.92	9.9+-2.0719	12+-1.1155	13.5+-1.012
MCV	0	69+-6.6594	70.1+-7.8619	70+-9.2461	71.3+-7.2148
	35	71.5+-8.4261	73+-8.4852	71.4+-10.2908	71.8+-7.0826
MCHC	0	32.7+-9.9186	36.7+-6.8351	36.6+-3.6029	39.3+-1.2499
	35	32+-5.2166	32.7+-o.9539	36.4+-3.6367	40+-1.3039
RDW	0	17.5+-1.4109	17.4+-1.7192	16.5+-2.0078	14.6+-1.5561
	35	17.7+-1.8839	17.3+-2.2108	16.2+-1.4689	15.4+-1.6854
MCH	0	25.9+-7.4228	26.1+-6.3942	25.4+-3.1240	27.9+-3.4064
	35	25.5+-5.3897	23.5+-3.1319	24.9+-5.0951	29.5+-4.4312

Table 4.6 Iron status of children enrolled in the study

	Day 0 n (%)	Day 35 n (%)
Normal	10 (27)	13 (35)
Iron deficiency*	2 (5)	3 (7)
Anemia with iron deficiency**	13 (34)	16 (42)
Anemia***	13(34)	6(16)

*Iron deficiency = MCV≤70, MCH < 27 and RDW≥16 but no anemia

** Iron deficiency +Anemia = hemoglobin <12gr/dL

*** Anemia with no iron deficiency = hemoglobin < 12g/dl

CHAPTER FIVE

5.0 DISCUSSION

This study found that 15 (39%) children aged 2 months to 5 years with malaria had blood film features of iron deficiency at the time of admission. Fifty percent of the children who had anemia (Hb < 12g/dl) and seventeen percent with normal hemoglobin had iron deficiency (MCV<70 fl, MCH<27 (pg) and RDW>16 %). Among the children with severe anemia (Hb<6 g/dl) who had mean corpuscular volume of less than 70 fl, only one child had a mean corpuscular volume of more than 70 fl on day 35 but still had a red cell distribution width of more than 16%. Children with moderate anemia (Hb 6<10g/dl) 87.5% had red cell distribution width of more than 16%, although only 3 had mean corpuscular volume of less than 70 fl. The red cell distribution width percentage of one child was less than 16 % on day 35 while the rest remained more than 16 % and higher. This could be due to increased erythropoesis or consumption of iron by the malaria parasite. The malaria parasite needs lots of iron for its own life cycle, and it manages to extract the iron from the host by inserting parasite-specific transferrin-like receptors on the host red cell membrane. Malaria parasites can therefore cause additional iron deficiency in the host [34] Some of the children with moderate anemia had low mean corpuscular volume and increased red cell distribution width. Four children had both low mean corpuscular volume and high percentage of red cell distribution width. The red cell distribution width improved in 2 children while in the other two it got worse on day 35. Four children with normal red cell distribution width

on day 0 had an increased value on day 35. In the group with normal hemoglobin (Hb > 12g/dl) there were only three children with low mean corpuscular volume and two with high percentage of red cell distribution width. However on day 35, five children had high red cell distribution width and those children with low mean corpuscular volume had a low value on day 35 with a reduction in hemoglobin. The P- Values for MCV, MCH, and RDW between day 0 and day 35 were 0.4224, 0.5420 and0.1805 respectively and were not statistically significance. The P-Values for Hb and RBC were 0.000762 and 0.000628 respectively and were statically significant.

Studies done in Africa to determine iron deficiency by giving iron prophylaxis and observing the improvement in hemoglobin in the general population had conflicting results. A study done at Macha Mission Hospital in the Southern part of Zambia by P Thuma et al showed that there was no significant difference in hemoglobin increase in children who were given iron prophylaxis as compared to those given placebo. The study was done at the end of malaria season May to August. The conclusion was that malaria is the main cause of anemia in the children from villages surrounding Macha Mission Hospital [35]. This study was done on a rural population and can not be a representation of the Zambian population. However in a Meta analysis of 12 published and unpublished placebo controlled iron supplementally trials done by Shankar et al, in the iron supplementally groups, the hematological improvements over the studies were significant and included an average increase of levels of 1.2 g/dl and a 50 % decrease in the risk of severe anemia. [27]. In this study, the average increase of hemoglobin in children with either low mean corpuscular volume or high percentage of red cell distribution width 1.5 g/dl where as those with normal indices was 1.78 g/dl. In a study

done among the Jewish and Bedouin toddlers to evaluate the levels of indices of iron deficiency anemia in their second year of life, there was significant rise of hemoglobin among children with abnormal indices after treatment with iron. [36] In this study no intervention was done and children who received blood transfusion were not included in the analysis for average increase in hemoglobin.

CHAPTER SIX

5.0 CONCLUSSION

This study shows that a third of children admitted to hospital in Zambia with malaria have iron deficiency anemia. The extent of the problem needs to be established in a bigger study where even the golden standard of diagnosing iron deficient anemia can be used. The limitations of this study are that other iron deficiency indices such as ferritin, trasnferin receptors, zinc protoporphyrin and serum iron levels could not be evaluated. In addition, the sample size was small. Therefore the results can not be extrapolated or be used to make decisions or change the practice in the management of malaria. Further studies need to be done to decide whether iron prophylaxis after malaria episode with anemia would be beneficial.

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Appendix 1

QUESTIONNAIRE FOR STUDY PATIENT CLINICAL ASSESSMENT

STUDY ID NUMBER. **DD MM YY** **FILE No. .....**

Date of admission to UTH. ..... Time .....

Date of discharge from UTH. ..... Date of Review. .....

Age ..... Sex ..... Religion. .....

Body weight..... Temperature on admission. .....Height. .....MAC. .....

Duration of fever . < 1 week. ..... >1 week. .....

Vomiting. ..... Diarrhoea. ..... Convulsion. .....

Previous blood transfusion. ..... Anemia. ..... Bleeding disorder. .....

Previous history of taking haematinics in the past 1 month. .....

Any other known illnesses. .....

PHYSICAL SIGNS.

Pallor. ..... Jaundice. ..... Lymphadenopathy. .....

Haemoglobinuria. ..... Splenomegally. ..... Hepatomegally. .....

Petachie. ..... Heart Failure. .....

Appendix 2

LABORATORY DATA FORM.

Patient's Id Number: ..... File No. .....

HEMATOLOGICAL PROFILE.

WBC ..... HB.....HCT.....RBC.....PLT .....

MCV.....MCH.....MCHC.....ESR.....MPV..... .... RDW.....

ANEMIA STATUS.

Blood Type ..... Serum Iron.....

Transferin Proteins..... Serum Ferritin.....

Erythropoetin.....

HEMOGLOBINOPATHY STATUS.

Sickling Test.....Hemoglobin Electrophoresis.....

Appendix 3

BUDGET.

ITEM.	**INCOME. (K)**	**EXPENDITURE. (K)**
Income		9,900,000
Less Expenditure.		
Administration/stationary	2,000,000	
Part time research assistant and labTecknowlogist	3,000,000	
Reagents and specimen bottles.	3,000,000	
Data analysis.	1,000,000	
Contingence.	900,000	
Total expenditure		9,900,000

Appendix 4

CONSENT FORM PARTICIPATION IN RESEARCH (ENGLISH)
(For children with uncomplicated malaria or malaria anemia)

You are invited to participate in a research project entitled: **prevalence of iron deficiency anemia in malaria.** If you agree, the following **tests or procedures will be performed** on your child:

--- Blood samples will be obtained for the purpose of blood counts, malaria parasite counts, tests to find out the cause of anemia, and tests that study the body's response to infection.
--- A stool sample will be obtained and examined for the presence of parasites.
--- A urine sample will be obtained and examined for the presence of parasites and infection.

Explanation to Participants

Dr Evans Mulendele is conducting a research project that investigates why new blood cells are not made properly by the body in some children with malaria. This research is also designed to see the children with malaria have low levels of iron which may predispose them to have severe malarial anemia. I would like to study about 150 children less than five years of age. Of these 150 children, 75 children will have severe malarial anemia, 75 children will have malaria with mild anemia.
For the purpose of the study, we will draw approximately two teaspoons of blood from your child today, and two teaspoons of blood again thirty-five days from today. The procedure involves placing a needle in your child's vein to take blood and will require about five minutes of your time. We will clean off the skin with alcohol before the procedure. We will also take a few drops of blood from a needle stick in your child's finger. Occasionally, minor complications, such as bruising, swelling, infection, and/or black and blue marks develop at the site where blood was drawn. A doctor, nurse, or clinical officer who are involved in this study and is familiar with this technique will draw your child's blood sample. We will also collect samples of your child's stool and urine and check them for the presence of parasites and/or infection.
After discharge from the hospital, we ask that you bring your child back to UTH for check-ups in five weeks (35 days from admission date). We will obtain another blood sample for research at the five week visit. Overall, we will draw a total of about 4 teaspoons of your child's blood, two teaspoons at the first visit, and two teaspoons at the five week (35 day) visit.

We believe that knowledge gained from this study will help us find better ways to treat and prevent malaria and anemia. There may be no direct benefit to your child for participation. You will receive nothing for any inconvenience that you may incur as a result of your child's participation in the study. You will not be charged for any of the tests and/or procedures to be performed.

Use of my Child's Blood Sample in Other Projects
[If I do not wish for my child's blood or mouth swab sample to be used in future IRB approved research studies, I will check the "NO" line below.]

☐ YES

☐ NO Initials: _____

I further understand that even if I checked "YES" above, but later change my mind and decide that I do not want you to use these samples for future tests, I can notify you and the samples will be destroyed.

Use of my Child's Blood Sample to test for the presence of HIV infection
[If I do not wish to have my child's blood tested for HIV, I will check "NO" below.]

You have my permission to test my child's blood for the presence of HIV (Human Immunodeficiency Virus) infection that causes AIDS. I understand that if I check "YES", the trained HIV/AIDS counselors at UTH will give me pre- and post-counseling for this test. The results of the test will be available to me at the time of the Day 35 review. I understand that this sample will be coded and not have my child's name on it, nor will the result of the HIV test be placed in the medical record or reported to anyone else including any governmental agency.

☐ YES

☐ NO Initials: _____

Participant's Statement of Understanding

I understand that in the event of physical or other injury resulting from the research tests or procedures, emergency medical treatment will be provided, but financial compensation will not be available.

A. I understand that participation in this project does not involve any risks other than those already described above. With this knowledge, and the above description of the project, I voluntarily agree for my child to take part and accept the risks of my child's participation in this research. Precautions have and will be taken to reduce the risks and to provide for my child's care.

B. I am aware that I am free to withdraw this consent and discontinue participation in this project at any time without affecting my relationship with the University of Zambia Teaching Hospital, or Yale University School of Medicine. I understand that if I withdraw from the study, the treatment will not be compromised in any way and all future hospital treatment/consultations will be not compromised either.

C. The University of Zambia Research Ethics Committee, and the Yale University School of Medicine Human Investigations Committee will have permission to look at the records of this project.

D. Dr. Evans Mulendele can be contacted at the Department of Child Health and Paediatrics, UTH. If, at any time, I have questions that I would like to discuss with someone other than the investigators on this project, I am free to talk to the Secretary of the UNZA Research Ethics Committee. I should contact Dr. Evans Mulendele in the event of a research-related injury.

I have read the above description of the project. Anything I did not understand was explained

to me by _____ and my questions were answered to my satisfaction. I was informed that my child would receive appropriate treatment for malaria whether I decide to enroll in this study or not. I agree to have my child participate in the **prevalence of iron deficiency anemia in malaria** research project.

Authorization:

I have read this form and decided that _____ will
(name of subject)
participate in the project described above. Its general purposes, the particulars of involvement and possible hazards and inconveniences have been explained to my satisfaction. My signature or thumbprint below also indicates that I have received a copy of this consent form.

Signature:_____ or Thumbprint

Relationship:_____

Date:_____

_____ _____
Signature of Primary Investigator Date

or

_____ _____
Signature of Person Obtaining Consent Date

If you have further questions about this project or if you have a research-related problem, you may contact the study doctor, Dr. Evans Mulendele at UTH. If you have any questions concerning your rights as a research subject, you may contact the University of Zambia Research Ethics Committee,

This was the consent form used also for the small study since it was nested in the big study.