PLASMA ZINC AND SEVERITY OF MALARIA IN CHILDREN LESS THAN FIVE YEARS OF AGE AT MUHIMBILI NATIONAL HOSPITAL, DAR ES SALAAM, TANZANIA.

By

Dr. Mike J Mabimbi, MD (DAR).

A dissertation submitted in partial fulfilment of the requirements for the degree of Masters of Medicine (Paediatrics and Child Health) of the University of Dar es Salaam.

University of Dar es Salaam.

September 2003.

CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the University of Dar es Salaam a dissertation entitled: *Plasma zinc and severity of malaria in children less than five years of age at Muhimbili National Hospital,* in partial fulfilment of the requirements for the degree of Masters of Medicine (Paediatrics and Child Health).

.....
PROFESSOR. KARIM MANJI.

(SUPERVISOR)

.....
PROFESSOR. AMOS MASELLE.

(SECOND SUPERVISOR)

DATE 26th September, 2003

DECLARATION

AND

COPYRIGHT

I, Dr. Mike Jewenanke Mabimbi declare that this dissertation is my own original work and that it has not been presented and will not be presented to any other University for a similar or any other degree award.

Signature .....

This dissertation is copyright material protected under the Berne Convention, the Copyright Act 1996 and other international and national enactment, in that behalf, on intellectual property. It may not be reproduced by any means, in full or in part, except for short extracts in fair dealing, for research or private study, critical scholarly review or discourse with an acknowledgement, without written permission of the Directorate of Postgraduate studies, on behalf of both the author and the University of Dar es Salaam.

ACKNOWLEDGMENT

I would like to acknowledge my supervisors Prof. A. Maselle and Prof. K. Manji for the unceasing guidance and encouragement at every stage of my work. My sincere gratitude to all members of Department of Paediatric and Child Health, especially Dr. F. Kalokola (the Head of Department) for their valuable suggestions.

I express my appreciation to SAREC (Swedish Agency for Research Cooperation with Developing Countries) for their financial support received through Malaria Project of MUCHS without which this work would not have been possible. I am grateful to Dr. G. Kwesigabo for his guidance and critical review of this work. I wish to thank Mr. C Makwaiya, Ms. C. Moshiro and Ms. R. Mpembeni for the assistance in statistical work. This work would not have been accomplished in time without the tireless efforts of Mr. J. Phillip, the Chemical analyst from the University of Dar es Salaam. I appreciate his help. I am grateful to all staffs from the Specialized Paediatric Laboratory (SPL) of MNH for the processing and storage of plasma zinc samples. I am indebted to the parents/guardians who consented for their children to participate into this study.

I express my appreciation in a very special way the contributions made by Dr. E. Munubhi, Dr. M. Fataki, Dr. T. Kazimoto, Dr. A. Massawe, Dr. J. Kitundu and Dr. M. Msomekela for their valuable comments. I would also like to thank Prof. G. Msamanga and Prof. Z. Premji for providing me with several useful references. Many thanks go to my loving family, brothers, sisters and friends for their understanding, their moral and material support.

DEDICATIONS

This work is dedicated to my late father Mabimbi.

Also,

To my mother Polina and my beloved wife Jamilla.

To our daughter and sons.

ABSTRACT

Background

Malaria is a leading cause of morbidity and mortality in Dar es Salaam. It has undergone a dramatic resurgence in recent years and children less than five years of age are the most vulnerable. The role of zinc and its severity has not been elucidated. Oral zinc supplementation has been shown to reduce substantially the episodes of severe malaria and frequency of health facility attendance.

Study Objective

The overall objective of this study was to assess plasma zinc levels and risk factors associated with severity of malaria in children less than five years of age admitted at Muhimbili National Hospital and those attending a Maternal and Child Health Clinic (MCH) of city council of Dar es Salaam, Tanzania.

Study design

It was an unmatched case control study with a ratio of 1:2 (one case with two arms of controls).

Study setting

This study was conducted in the General Paediatric wards, General Paediatric Outpatient clinic of MNH and at Mnazi Mmoja MCH clinic of Dar es Salaam city council.

Subjects

A total of 150 children (56.6% males and 43.4% females) aged 1 to 59 months were enrolled into the study from July to October 2002. The study had 50 severe malaria cases with 100 controls of two arms, 50 with uncomplicated malaria and 50 well children.

Methodology

Eligible children were enrolled for the study after meeting inclusion criteria and parents/guardians giving an informed consent. A questionnaire was used to record history, physical findings and all laboratory results. Venous blood sample of 4 mililitres was drawn and plasma obtained within 30 minutes before being deep-frozen at –20 degrees centigrade. Plasma zinc analysis was performed within 2 weeks at the department of Chemistry of the University of Dar es salaam, using *Standard Atomic Absorption Spectrophotometry (SAAS)* for Zinc.

Plasma zinc level *<10 μmols/L* was considered as Plasma zinc deficiency.

Main outcome

Plasma zinc levels in each of three groups of children studied (severe, uncomplicated and children without malaria).

Results

Ninety-six (66%) out of all 150 children had low plasma zinc levels. The levels were inversely proportional to malaria severity (the lowest plasma zinc levels found in most severe malaria cases). Forty two out of 50 (84%) among children with severe malaria, twenty-nine out of 50 (58%) among uncomplicated and twenty-eight out of 50 (56%) among children without malaria had low plasma zinc levels. The mean plasma zinc levels was much lower in severe malaria group, 7.019 μmols/L (±4.052) compared to 11.031 μmols/L (±5.43) and 12.490 μmols/L (±8.310) for uncomplicated and without malaria children respectively. The differences were statistically significant. The odds ratio of low plasma zinc levels among children who had uncomplicated malaria compared to those who had low plasma zinc levels among children with severe malaria was 3.8 (95% CI 1.5,10) with a p-value of 0.007. The odds ratio of low plasma zinc levels among children without malaria and low plasma zinc levels among children with severe malaria was 4.1(95% CI 1.6,11) with a p-value of 0.004. ITNs users had a higher plasma zinc levels and decreased malaria severity. The odds ratio for low plasma zinc levels on use of ITNs between severe and uncomplicated malaria was 0.1 (95% CI 0.01, 0.6) with a p- value of 0.002.

The odds ratio for low plasma zinc levels on use of ITNs between severe and no malaria group was 0.12 (95%CI 0.01,1.1) with a p-value of 0.005. The differences were statistical significant.

Age, sex and nutritional status were not risk factors associated with low plasma zinc and increased malaria severity.

Conclusion

- Low plasma zinc levels of <10 μmols/L were significantly correlated to the severity of malaria. This information, may serve as a useful intervention to control severity of malaria morbidity and mortality.

Recommendation

- The study provides useful baseline information that plasma zinc levels are altered in malaria however there is a need for large studies (prospective cohort) to elucidate the causal relationship of zinc and malaria infection and recommend public health measures for zinc supplements.

TABLE OF CONTENTS

Item **Page**

TITLE i

CERTIFICATION.....ii

DECLARATION & COPYRIGHT.....iii

ACKNOWLEDGMENT.....iv

DEDICATIONS.....vi

ABSTRACT.....vii

LIST OF TABLES xiv

ABBREVIATIONS xv

1.0 INTRODUCTION AND REVIEW OF LITERATURE.

1.1 Epidemiology of Malaria

1.1.1 Introduction.....1

1.1.2 Malaria situation globally.....2

1.1.3 Malaria situation in Tanzania.....4

1.1.4 Malaria situation at MNH.....6

1.2 Pathology of malaria

1.2.1 Pathological diagnosis of malaria.....7

1.2.2 Case definitions of malaria 9

1.2.3 Clinical manifestations of severe malaria.....10

1.2.4 Malaria treatment in Tanzania.....13

1.3 Plasma zinc

1.3.1 Role of zinc in human body.....14

1.3.2 Zinc homeostasis.....16

1.3.3 Nutrient interactions.....16

1.3.4 Epidemiology of zinc deficiency.....18

1.3.5 Associations of Plasma zinc deficiencies and malaria.....19

1.3.6 Associations of Plasma zinc deficiencies and other diseases.....21

2.0 RATIONALE AND OBJECTIVES OF THE STUDY.

2.1. Rationale.....25

2.2. Hypothesis.....26

2.3. Broad objective.....26

2.4 Specific Objectives.....27

3.0 METHODOLOGY.

3.1 Study design.....28

3.2 Study setting.....28

3.3 Study population and inclusion criteria.....29

3.4 Exclusion criteria.....30

3.5 Sample size estimation.....30

3.6 Study duration.....31

3.7 Sampling technique and recruitment.....32

3.8 Data collection

3.8.1 Demographic data.....32

3.8.2 History/Physical examination.....33

3.8.3 Blood slide for malaria parasites.....33

3.8.4 Haemoglobin examination.....34

3.8.5 Plasma zinc examination.....35

3.9 Ethical Issues/ Clearance.....36

3.10 Management and disposal of participants.....37

3.11 Quality assurance.....37

3.12 Data analysis.....38

3.12 Limitations of the study.....38

4.0 RESULTS.....39

5.0 DISCUSSION 48

6.0 CONCLUSIONS.....55

7.0 RECOMMENDATION.....56

8.0 REFERENCES.....57

9.0 APPENDICES

9.1 Appendix I – Questionnaire.....63

9.2 Appendix II - written consent- English version.....66

9.3 Appendix III - written consent- Swahili version.....69

LIST OF TABLES

Table number **Page**

Table 1: A table of population distribution of children according to age and sex.....39

Table 2: A table of association between plasma zinc levels and malaria severity.....40

Table 3: A table of association between plasma zinc levels and malaria severity on age groups.....42

Table 4: Table of association between plasma zinc levels and malaria severity on sex groups.....43

Table 5: A table of association between plasma zinc levels and malaria severity by use of ITNs.....45

Table 6: A table of association between plasma zinc levels and malaria severity on nutritional status by WFA.....46

ABBREVIATIONS/ACCRONOYMS

AIDS- Acquired Immuno Deficiency Syndrome

ANOVA- Analysis of Variances

ARDS- Acute Respiratory Distress Syndrome

CI- Confidence Interval

CSF- Cerebro-Spinal Fluids

CuZnSOD- Copper-Zinc Superoxide Dismutase

DNA- Deoxy Ribonucleic Acid

EDTA- Ethylene Diamine Tetra Acetic Acid

ESR- Erythrocyte Sedimentation Rate

G6PD- Glucose- 6- Phosphate Dehydrogenase

GPW- General Paediatric Ward

HIV - Human Immune Deficiency Virus

IGF-1- Insulin like - Growth Factor-1

ITN- Insecticide Treated Net

IPT- Intermittent Presumptive Treatment

JICA- Japan International Cooperation Agency

MCH- Maternal and Child Health

MMed- Masters of Medicine

MOH - Ministry of Health

MNH- Muhimbili National Hospital

MUCHS- Muhimbili University College of Health Sciences

OPD- Out patient Department

OR- Odds Ratio

PCD- Programmed Cell Death

PCV- Packed Cell Volume

PEM- Protein Energy Malnutrition

RBC- Red Blood Cell

RDS- Respiratory Distress Syndrome

SAAS- Standard Atomic Absorption Spectrophotometry

SP- Sulphadoxine Pyrimethamine

SPL- Specialized Paediatric Laboratory

TNF- Tumour Necrotic Factor

Uncompl-Uncomplicated malaria

WFA- Weight for Age

WBC- White Blood Cell

WHO- World Health Organization

Zn- Zinc

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 Epidemiology of Malaria

1.1.1 Introduction

Malaria is a parasitic disease caused by plasmodium species. The four plasmodium species are *P. falciparum, P. vivax, P. ovale* and *P. malariae.* The last three species don't cause severe malaria but cause an intermittent fever and chills and are usually not debilitating. *P falciparum* on the other hand accounts for the severe type of malaria and is lethal. *P. falciparum* has multiple organ affection that include the brain, liver, renal and lungs. It is the most common type seen in Africa south of Sahara.[1]

Female anopheles mosquito transmits malaria parasite. Transmission of malaria is affected by climate and geography that coincide with rainy season. During rain seasons there are maximal breeding sites. The female anopheles mosquito spread the disease by biting an infected individual and transmits to the uninfected individual after undergoing several stages in the female mosquito.[2] Children less than five years of age are more vulnerable to the disease because they have not yet developed immunity to the parasites. Again their small underdeveloped bodies are particularly sensitive to the effects of malaria whereby affected children can die within 48 - 72 hours of developing symptoms.

Children less than five years of age usually get from 4 – 6 bouts o clinical malaria in a year.[1]

1.1.2 Global Malaria situation

In Africa 90% of malaria cases are caused *by P. falciparum.* About 25% of childhood deaths in paediatric care units in Africa are caused by malaria. Each year there are on average of 300,000 to 500,000 new malaria cases.[3] About 90 countries or territories worldwide are considered risk geographic areas for malaria and 90% of these are located in the African continent. This means that 35% of total world population are at risk of acquiring malaria.[3] According to WHO report malaria is one of the planet's deadliest disease and one of leading causes of sickness and death in the developing world. Out of 500 million clinical cases of malaria each year 1.5 to 2.7 million deaths occurs worldwide. Eighty to ninety percent of malaria deaths occur in sub Sahara African where 90% of the infected people live.[4] Malaria is responsible for as many as half of the deaths of African children less than five years of age. Malaria kills more than 1 million children (2,800 per day or 2 children per second) each year in Africa alone. [4] Globally it is estimated that 275 million out of 530 million people carry malaria parasites in their blood, although they may not develop symptoms.

Among the four human malaria strains, *P. Falciparum* is the most common and deadly form, responsible for about 95% of malaria deaths worldwide and mortality rate of 1- 3%. [4] In early 1960s, only10 % of the world's population was at risk of contracting malaria. This rose to 40% as mosquitoes developed resistance to pesticides and malaria parasites developed resistance to treatment drugs. Malaria is now spreading to areas previously free of the disease.

Malaria in terms of expenditure:

An estimated global annual direct and indirect cost including lost labor in 1995 was 2 billion dollars. The worldwide estimated expenditure on malaria research prevention and treatment was 84 billion dollars. The estimated worldwide expenditure per malaria fatality in 1990 was 65 dollars compared to 3, 374 dollars for HIV/AIDS and 789 dollars for asthma. This is to say, 50 malaria deaths equals one HIV/AIDS death. [5]

Malaria in terms of economic effects:

Malaria is a disease of the poor and the world's poorest people living in rural communities are affected. Highly malarious countries are among the poorest in the world and typically have low economic growth. [4]

The socio-economic effects have been devastating. According to a recent study at Harvard University malaria has the effects of slowing down economic growth by 1.3% in a year.

Over last 30 years Africa has spent at least 100 billion dollars due to malaria.[5] The morbidity and disability associated with malaria is huge. Rural and agricultural communities where the majority of Africans populations live are particularly hit hard by malaria. The intensity of malaria transmission often coincides with the rainy season, a time when agricultural activity is at its peak therefore leading to increase in lost days of works and aggravating rural poverty.[4]

1.1. 3 Malaria situation in Tanzania

Thirty million people are at risk of being infected with malaria. Sixteen million get acute illness with 100,000 deaths annually. Most malaria deaths occur in children less than five years of age and pregnant women. Malaria has spread to involve highlands of Kilimanjaro, Usambara and Rungwe. In terms of patient load, the situation has worsened from 11-13% in 1978 to 34% in 1997.[7] Malaria situation in Tanzania has not improved since David Clyde in 1967 produced the map of distribution of malaria in Tanzania, for the most part the situation has deteriorated.

Since the founding of health services in Tanzania, malaria has been a leading cause of morbidity and mortality in the country. It is generally agreed that malaria is the number one cause of hospital attendance in Tanzania. Malaria accounts for 15% of all hospital attendances and 10% of all admissions. [6] Malaria is a leading cause of death in hospitals. There is a great variation in level of malaria endemicity in Tanzania. The variations are due to human habits, parasite characteristics, climate, topography, vegetation and land use. Mosquito habits and habitats also contribute the levels of malaria endemicity. There are three malaria patterns in Tanzania.[7]

- Unstable seasonal malaria; where transmission is less than three months per year. This is seen in areas of 2000m above sea levels like Mbeya, Iringa, Hanang and Kilimanjaro.
- Stable malaria with seasonal variation; where transmission ranges from 3-6 months per year. This is commonly found in Dodoma, Singida, Kigoma and Ruvuma regions.
- Stable perennial malaria; where transmission ranges from 6-12 months in a year. This is seen in Coastal belt areas of Dar es Salaam, Coast, Morogoro, Lindi and regions around Lake Victoria and Nyasa.

The major vector in Tanzania is *Anopheles gambiae* complex. All species that affect man occur in Tanzania. *P. falciparum* accounts for 90% of the infections. Therefore malaria in Tanzania mainly is a disease due to *P. falciparum* infections.[6] The other three species account for chronic infections. *P. malariae* has a 10% of all infections (usually mixed with P. falciparum), *P. ovale* less than 5% while *P. vivax* is extremely rare although it used to be prevalent in the past.[6]

1.1.4 Malaria at Muhimbili National Hospital

The General Paediatrics Wards of MNH admits an average of 800 patients in a month, One third of all admissions have positive blood slides for malaria parasites. The commonest presenting features include; fever, malaise, joint pains and cough in the uncomplicated malaria. Severe malaria accounts for 9-10% of all cases of malaria. [8] Among cases for severe malaria, severe anaemia predominates 49% while congestive cardiac failure is 15.5%, respiratory distress is (11.3%) and cerebral malaria is (9.3%). Eighty percent of all malaria cases in children are from 6 months to 5 years of age.[8] Morbidity trend for malaria has increased by 8 folds while mortality rate has risen by 6 folds. Malaria is affecting more children today than used to be 25 years ago as noted from escalating trend in this hospital. [8]

1.2 Pathology of Malaria

1.2.1 Pathological diagnosis

The most pronounced pathological changes related to malaria involve the blood and the blood forming system, spleen and the liver. Secondary changes can occur in all other major organs, depending on the type and severity of the infection. The pathological changes are more profound in case of P. falciparum malaria.[9] The RBCs are the principal sites of infection in malaria. The clinical manifestations are due to the involvement of RBCs. The growing parasite consumes and degrades the intracellular proteins mainly heamoglobin. The transport properties of RBC membrane are altered, cryptic antigens are exposed and new parasite derived proteins are inserted. The RBC becomes more spherical and less deformable. In P. falciparum infection, membrane protuberances appear on the red cell surface in the second 24 hrs of the asexual cycle.[10] Accretions of electron dense, histidine - rich parasite proteins are found under these 'knobs'. The knobs extrude a strain specific, adhesive variant protein of high molecular weight that mediates red cell attachment to receptors on venular and capillary endothelium, causing cytoadherence.[9]

P. Falciparum infected RBCs also adheres to uninfected RBC to form rosettes. The cytoadherence and rosetting are central to the pathogenesis of *P. falciparum* malaria. The formation of RBC aggregates and intravascular sequestration of RBC in vital organs such as brain and the heart interferes with the micro-circulation and metabolism and allows parasite development away from the principal host defense, spleenic processing and filtration.[11] Due to this reason in *P. falciparum* malaria only younger forms of the parasite are found in the peripheral circulation and the peripheral parasitemia is usually an underestimate of the true parasite load.[11]

Mature forms of *P. falciparum* are rarely seen in the peripheral blood and when found, indicate severe infection. Sequestration does not occur in cases of *P. vivax* and *P. malariae* infections and therefore, all stages of the parasite can be seen in the peripheral blood and complications are very are.[11] Leukocyte count is either low or normal in most cases of malaria. Increase leukocytes count indicates either a severe infection or secondary bacterial infection. Reduction in the leukocyte count is attributed to hyperspleenism or sequestration in the spleen. Relative lymphocytosis, monocytosis, eosinopenia and presence of stab neutrophils is observed with prolonged duration of the illness.[9-11]

Thrombocytopenia is also common in malaria and this may be related to sequestration of the platelets in the spleen. Severe thrombocytopaenia however indicates severe infection and may herald bleeding syndromes. ESR is usually elevated in malaria up to 30 – 50 mm in the first hour. Prolonged and severe malaria along with severe anaemia are usually associated with a higher ESR. [11]

1.2.2 Clinical case definitions (by WHO/MOH guidelines)

Diagnosis without laboratory access: [12]

- Probable uncomplicated malaria: Person with symptoms and/or signs of malaria who receives anti malarial treatment.
- Probable severe malaria: A patient who requires hospitalization for symptoms and signs of severe malaria and receives antimalarial treatment.
- Probable malaria death: Death of a patient diagnosed with probable severe malaria.

Diagnosis with laboratory access: [12]

- Asymptomatic malaria: A person with no recent history of symptoms and/or signs of malaria who shows laboratory confirmation of parasitemia.

- Confirmed uncomplicated Malaria: A patient with symptoms and signs of malaria who received antimalarial treatment, with laboratory confirmation of diagnosis.
- Confirmed severe malaria: A patient who requires hospitalization for symptoms and/or signs of severe malaria and receives antimalarial treatment, with laboratory confirmation of diagnosis.
- Confirmed malaria death: Death of a patient diagnosed with severe malaria, with laboratory confirmation of diagnosis.

Malaria treatment failure: A patient with uncomplicated malaria without any clear symptoms suggesting another concomitant disease, who has taken a correct dose of antimalarial treatment and who presents with clinical deterioration or recurrence of symptoms within 14 days of the start of treatment in combination with parasitemia

1.2.3 Manifestations of severe malaria

- Cerebral malaria with convulsions and coma. [12-13]

Deep unarousable coma. Coma should persist for more than 30 minutes after generalized convulsions. Encephalopathies from other causes should be excluded like hypoglycemia, meningoencephalitis, eclampsia, intoxication, injuries, cerebral vascular accidents and metabolic disorders.

Confirmation of P. falciparum infection by isolation of asexual forms in peripheral blood or bone marrow during life or in brain smears after death.

- Severe anaemia: Haematocrit less than 15% (Haemoglobin less than 3.1 mmols/L or 5g/dl).
- Jaundice: Serum bilirubin of more than 50 μmol/L (3mg/dl).
- Renal failure: Urine output < 400 ml in 24 hrs or <12 ml/kg/24 hours in children and a serum creatinine of > 265 mmol/L (> 3.0 mg/dl), failing to improve after rehydration.
- Pulmonary oedema or ARDS: Breathlessness, bilateral crackles and other features of pulmonary oedema.
- Hypoglycemia: Blood glucose concentration < 2.2 mmol/L (less than 40 mg/dl).
- Hypotension and shock: Systolic BP <50 mmHg in children 1 - 5 years or <80 mmHg in adults; core skin temperature difference $>10^0$ C.
- Bleeding and clotting disturbances: Significant bleeding and haemorrhage from the gums, nose, gastrointestinal tract, retinal haemorrhage and/or evidence of disseminated intravascular coagulation.

- Hyperpyrexia: Rectal temperature above ≥ 39.5^0 C.
- Fluid, electrolyte or acid base disturbances requiring intravenous fluid therapy, arterial pH <7.25 or plasma bicarbonate <15 mmols/L and venous lactate > 6 mmols/L.
- Haemoglobinuria: Macroscopic black, brown or red urine; Must not be associated with effects of oxidants drugs or enzymes like G6PD deficiency.
- Hyperparasitemia; A parasite density of asexual forms of *P. Falciparum* in the peripheral smear of ≥ 10,000 asexual per μL of blood.
- Complications or associated infections: Aspiration bronchopneumonia, septicemia and urinary tract infection. Vomiting of oral treatment. Patient with persistent vomiting may have to be admitted for parental therapy.
- Impaired consciousness: Various levels of impairment in the level of consciousness may indicate severe infection although not falling into a definition of cerebral malaria. These patients are generally arousable.
- Extreme generalized weakness, prostration and dehydration that need support.

- Convulsions: More than two generalized seizures in 24 hours with regaining of consciousness.
- Other indicators of severity and poor prognosis include: [12,13]

  Leukocyte count > 12,000/mm3

  High CSF lactates, low CSF glucose

  Low anti-thrombin III levels

  Peripheral schizontemia.

1.2.4 Malaria Treatment in Tanzania

Malaria is a curable disease with a wide range number of drugs. In 2001, a widespread on chloroquine resistant in Tanzania necessitated the National malaria control program to introduce SP (Sulfadoxine Pyrimethamine) as a first line treatment for uncomplicated malaria.

Uncomplicated Malaria: The first line drug of choice is a combination of sulfadoxine and pyrimethamine (SP). SP is an antifolate with synergistic effect highly against blood schizonticide of P. falciparum.

Sulfadoxine and pyrimethamine is also indicated in Intermittent Presumptive Treatment in pregnancy (IPT). [15] The second line drug for uncomplicated malaria is Amodiaquine, a 4 –aminoquinoline of similar structure to chloroquine. Amodiaquine also possesses an antipyretic and anti-inflammatory quality.

Another indication for amodiaquine is for treatment of uncomplicated malaria where SP is contraindicated.[14]

Severe Malaria: The drug of choice is parenteral Quinine given preferably by intravenous infusion. Quinine is an alkaloid derived from the bark of the cinchona tree. It is a blood schizonticide. Other indication apart from severe *P. falciparum* malaria includes multi drug resistant malaria. Infusion should be discontinued as soon as the patient is able to take oral medication. None response to Quinine (failure to clear parasite after three days of treatment should be treated with one of the Artemisinin derivatives along with quinine. [14]

1.3 Plasma zinc

1.3.1 Role of zinc in human body

Zinc (Zn) is an essential trace element for all forms of life. It is generally non toxic and is essential for normal growth, development, wound healing, immunocompetence and reproductive system.

Zinc plays a leading role in the production of enzymes, hormones and other substances helping to regulate growth activity, development and functioning of the immune and reproductive systems. [15] Males are said to be more susceptible to zinc deficiency but respond more readily to supplementation. [16] At cellular level zinc has a catalytic, structural and regulatory function.

Catalytic functions: Nearly 100 different enzymes depend on zinc for their ability as catalyst in many vital biological reactions. Zinc dependent enzymes can be found in all known classes of enzymes. [16, 17]

Structure functions: Zinc plays an important role in structure of proteins and cell membranes. A finger like structure, known as zinc motif, stabilizes the structure of a number of proteins. Copper provides the catalytic activity for the antioxidant enzyme copper-zinc superoxide dismutase (CuZnSOD) where zinc plays a critical structural role.[17] The structure and function of cell membranes are also affected by zinc.

Loss of zinc from biological membranes increases their susceptibility to oxidative damage and impairs their function.[15-18]

Regulatory functions: Zinc finger proteins have been found to regulate gene expression by acting as transcription factors (binding to DNA and influencing the transcription of specific genes). [17,18]

Zinc plays a role in cell signaling and has been found to influence hormone release and nerve impulse transmission. It has also been found to play a role in apoptosis (Programmed cell death - PCD). PCD is an important cellular regulatory process with implications for growth and development.[16, 18]

1.3.2 Zinc homeostasis

Zinc absorption is mainly in the proximal small intestines but the mechanism is unclear.[20, 21] Inhibition of inorganic zinc absorption is affected by iron and folate while dietary phytates decreases zinc absorption of both types (organic and inorganic type). [20] Zinc homeostasis is possibly regulated both by altering the efficiency of absorption, metal free albumin and secretion into intestines, the main route of its excretion. Each day about 14 mg of Zinc undergoes an enterohepatic circulation. In blood 80 % of zinc is in the RBCs. Almost all of this is in the carbonic anhydrase enzyme.[22] Thus with increased haemolysis zinc homeostasis will be disturbed. Nearly 3% of zinc is found in WBCs.

Each WBC contains approximately 25 times the amount of zinc as in each individual RBC. The rest, 17% of zinc is found in the plasma. In newborn RBCs levels are about half of that is of adult with levels progressively increasing until about12 years of age. [21]

1.3.3 Nutrient Interaction with Zinc (Copper, Iron, Calcium, Folate)

Copper: Large quantities of zinc (50 mg/day or more) over a period of weeks interfere with copper bioavailability.

High zinc intake induces intestinal synthesis of copper binding protein called metallothionein, which traps copper within intestinal cells and prevents its systemic absorption. [18, 20]

Iron: Supplemented iron in a dose of 38 - 65 mg/day elemental iron may decrease zinc absorption. It is an important phenomenon in management of iron supplementation during pregnancy and lactation and has led some experts to recommend zinc supplementation for pregnant and lactating women taking more than 60 mg/day of elemental iron.[16, 17]

Calcium: High levels of dietary calcium impair zinc absorption in animals but it is uncertain whether this occurs in humans. Calcium in combination with phytic acid reduces zinc absorption. Thus diets rich in phytates such as cabbages, wheat and corn may reduce zinc absoption.[18, 20]

Folic Acid: Bioavailability of dietary folate is increased by the action of zinc dependent enzymes, suggesting a possible interaction between zinc and folic acid.[16]

1.3.4 Epidemiology of zinc deficiency

Data regarding magnitude of zinc deficiency in Tanzania is lacking however it is considered a significant public health problem in children. In a study done in Dares Salaam as part of MMed dissertation in 1997 it was reported that 74% of all severe malnourished children admitted at the Nutrition centre (MNH-Makuti) had zinc deficiency. The mean plasma level on recruitment was 0.47 mg/l. [22]

Currently a study on zinc supplementation (in an endemic area) for children less than five years of age is being undertaken in Pemba and Zanzibar (Bob, USAID 2002). Tanzania Food and nutrition Centre (TFNC) is carrying out studies on zinc supplementation in three out of seven districts but not on zinc and malaria per se. There is evidence to suggest that 38-50% of all children in developing countries are zinc deficient. [23] Inadequate zinc intakes are highly prevalent in developing countries especially during the period of complementary feeding when zinc requirements are high and breast milk contribute little.[24]

Mild to moderate zinc deficiency may be relatively common worldwide but the public heath importance of zinc deficiency is not well-defined.[27] Zinc requirements for mothers during lactation are greater than those during pregnancy, especially in early weeks postpartum.

Phytates decrease zinc absorption hence the requirement is increased in children with a cereal-based diet. Large bolus doses of zinc are not effective because of lack of adequate storage capacity therefore small regular doses are more beneficial.[26]

1.3.5 Plasma zinc deficiency and malaria

Several studies have indicated that zinc supplementation may reduce the incidence of clinical attacks of malaria in children. Most recently placebo - controlled trial in preschool children in Papua New Guinea found that zinc supplementation reduced the frequency of health centre attendance due to *P. falciparum* malaria by 38%. In addition it was noted that high malaria parasitaemia was reduced by 68% following zinc supplementation. [28] Hence zinc supplementation may be of benefit in preventing malaria severity. In studies conducted in Malawi and Papua New Guinea that wanted to see any association between zinc status, anaemia and malaria concluded that zinc status was inversely correlated with P. falciparum malaria.[29]

In a study done in India using murine models it had shown that zinc deficiency was shown to exacerbate malaria parasitaemia, mortality and lipid peroxidation during infection.[30]

Early evidence from a study in Papua New Guinea suggests that zinc and vitamin A supplements may boost children's resistance to malaria.

In controlled trials, regular Vitamin A and zinc supplementation appeared complementary in decreasing the burden of malaria in children. Vitamin A reduced by more than a third the febrile illness due to mild to moderate high levels of malaria parasite in children. Zinc on the other hand helped reduce the severity of the worst malaria cases. As a result, the number of malaria cases seen at health centers given zinc was a third less than the number seen at health centers given a placebo. Overall clinic visits by these children who had received zinc decreased by a third and signs of other infections (cough or diarrhoea) were reduced by 20% to 50%. [34, 35]

Zinc supplementation reduced P. falciparum attributable health centre attendance by 30% and the impact was greatest after the first month of supplementation.[33] In another study that investigated micronutrient malnutrition and pathogenesis of anaemia due to malaria concluded that micronutrients such as vitamin A, vitamin E and Zinc may lower the morbidity of malaria through immune modulation and alteration of oxidative stress.[32] Another study on zinc and malaria reported that, zinc supplemented children had lower rates of diarrhoea, pneumonia and malaria in comparison with children not given zinc.[33]

Preliminary evidence suggested that zinc supplementation in children from developing country settings may also reduce infant mortality but large trials were needed to address this important issue conclusively.[33]

In another study of 50 malnourished children in1996 done in Tanzania it was found that iron, zinc and copper levels were not significantly different compared to normal children.[43] A multi-center study on zinc supplementation in children diagnosed with malaria carried out in Ecuador, Ghana, Tanzania, Uganda and Zambia, found no added benefit of zinc to the outcome of acute uncomplicated malaria. The study did not elucidate the zinc levels with severe malaria. [44]

1.3.6 Association of zinc deficiency with other diseases

Severe Zinc deficiency: Severe zinc deficiency came to be understood much better when individuals born with a clinical condition called *acrodermatitis enteropathica,* a genetic disorder resulting from impaired uptake and transport of zinc were diagnosed. Symptoms of severe zinc deficiency, which include slowing, or cessation of growth and development, delayed sexual maturation, characteristics skins rashes, chronic and severe diarrhoea. Other symptoms of the disease include immune system deficiency, impaired wound healing, diminished appetite, impaired taste sensation, night blindness, swelling and clouding of corneas and behavioral disturbances. [36, 37]

Before the cause of acrodermatitis enteropathica was known, patients typically died in infancy. Oral zinc replacement therapy results in complete remission of symptoms though should be maintained indefinitely in individuals with the genetic disorders.[37,38] Although dietary zinc deficiency is unlikely to cause severe zinc deficiency in individuals without a genetic disorder zinc loss, it can occur in severe burns or prolonged diarrhoea may also result in severe zinc deficiency. [36]

Mild Zinc deficiency: Recently it has become apparently that zinc deficiency contributes to a number of health problems especially common in children who live in developing countries. On the other hand lack of a sensitive indicator of mild zinc deficiency hinders the scientific study of its health implications.[36] Controlled clinical trials of moderate zinc supplementation have demonstrated that zinc deficiency contributes to impaired physical and neuropsychological development, and increases susceptibility to life threatening infections in young children.[36, 37]

Respiratory tract infections: More data shows that the incidence of acute respiratory tract infections and clinical attacks of malaria may also be reduced by zinc supplementation. Zinc supplementation is said to reduce the incidence of lower respiratory tract infection like pneumonia. [28.] In a pooled analysis study in developing countries it demonstrated a reduction in prevalence of pneumonia in children supplemented with zinc.[39]

Growth retardation, delayed neurological and behavioral development: Significant delays in linear growth and weight gain are common features of mild zinc deficiency in children. In randomized placebo-controlled study on zinc supplementation (Denver-USA, 1970s and 1980s) in young children with significant growth delays when given modest zinc supplementation of 5.7mg/day resulted in increased growth rates compared to placebo. [40] The exact mechanisms of the growth limiting effects of zinc deficiency are not known. Recent research indicates that zinc availability is involved in cell-signaling systems that coordinate the response to the growth hormones, insulin -like growth factor 1 (IGF 1). [41] Low zinc nutritional status has been associated with diminished attention in the newborn infants and poor motor function.

Adequate zinc intake is essential in maintaining the integrity of the immune system and zinc deficient individuals are known to experience increased susceptibility to a variety of infectious agents.[42]

2.0 RATIONALE AND STUDY OBJECTIVES

2.1 Study rationale

Zinc promotes normal growth and development and is an element in enzymes that works with RBCs to move carbon dioxide from tissues to lungs. It also maintains an effective immune system. In recent years malaria has undergone a dramatic resurgence due to a number of reasons including increasing resistance of the mosquito to insecticides, widespread resistance of the parasite to many anti malarial drugs, the breakdown of malaria control programs due to war and political instability and climate changes and natural disasters. Studies have shown that ITNs decreased the incidence of malaria infections and reduced hospital attendance. Increased frequency of malaria infection in absence of ITNs may deplete body zinc stores hence it would be appropriate to see the effect of ITNs and plasma zinc levels. It is possible that combinations of above factors and the deficiency of essential trace element zinc, could lead to a dramatic increase in the number of severe malaria cases. There is an association between zinc supplementation and the reduction of malaria severity in children.

Zinc supplements have been shown to reduce the most severe malaria cases in children less than five year of age.

Worldwide including Tanzania there are few studies on the relationship between malaria and plasma zinc. This study was conducted to document the different plasma zinc levels in children less than five years of age with and those without malaria and determine any association to malaria severity. Currently in Tanzania and other countries, zinc supplementation to decrease malaria morbidity and mortality in children less than five years of age is still being evaluated. Therefore this study will contribute additional information in that regard.

2.2 Hypothesis

A plasma zinc level below 10.0 μmols/L is associated with increased malaria severity in children less than five years of age.

2.3 Broad Objective

To assess plasma zinc and risk factors associated with severe malaria in children less than five years of age admitted in GPW at MNH Dar es Salaam, Tanzania.

2.4 Specific Objectives; Were to determine:

1. Plasma zinc levels in children less than five years of age with and those without malaria infections.
2. Association between plasma zinc levels and malaria severity according to age and sex.
3. Association between plasma zinc levels and malaria severity on use of insecticide treated bed nets (ITN).
4. Association between plasma zinc levels and malaria infections according to nutritional status using weight for age (WFA).

3.0 METHODOLOGY

3.1 Study design

It was a case control study, an unmatched type with ratio of 1:2.

3.2 Study setting

This study was conducted at three sites, the general paediatric wards (GPW), general paediatric OPD clinic of MNH and Mnazi Mmoja MCH clinic for children less than five years of age. The GPW contributed all malaria cases (n=50) and some uncomplicated malaria children (n=36). Fourteen (n=14) uncomplicated malaria children were recruited from general paediatric OPD. The general paediatric wards were chosen because laboratory investigation facilities were available.

The hospital has four general paediatric wards that admit children aged one month to seven years. The average admission in the general paediatric wards, according to Paediatric department report (2002) was said to be at least 800 per month. Children without malaria were recruited from Mnazi Mmoja MCH clinic. The Mnazi Mmoja MCH clinic was chosen because it is in the centre of Dar es Salaam city and had a laboratory facility suitable for blood slide malaria parasite, haemoglobin level examination and plasma storage.

Plasma zinc levels determinations were performed at the Chemistry department of the University of Dar es Salaam. Blood slides for malaria parasites and haemoglobin levels examinations were performed either at MNH or Mnazi Mmoja MCH clinic.

3.3 Study Population and inclusion criteria.

Cases; Were children:

- Aged 1 to 59 months of age admitted in general paediatric wards with a World Health Organization (WHO) clinical diagnosis of severe malaria.
- Who had any positive blood slide for malaria parasites and or blood slide of ≥ 10,000 asexual parasite/μL of blood.
- Who had not received blood transfusion in the past 4 months prior to recruitment.

Controls: First arm of controls- Uncomplicated Malaria; Were children:

- Aged 1 to 59 months attending General Paediatric OPD clinic or admitted in the GPW with a WHO clinical diagnosis of uncomplicated malaria.
- Who had a non- heavy malaria parasitaemia blood slide (of < 10,000 asexual parasites/μL of blood).

Second arm of control- Children without malaria:

- Aged 1 to 59 months attending Dar es Salaam city MCH clinic at Mnazi Mmoja for routine growth monitoring and immunizations.
- Normal temperature and normal respiratory rate according to age.
- Weight for age (WFA) ≥ 80%.
- Had negative blood slides for malaria parasites.

3.4 Exclusion criteria

- All children below 1 month of age.
- Unwillingness of the parents/guardians to participate in the study.
- Children who were jaundiced.
- Blood transfusion within the past 4 months.

3.5 Sample size estimation

Sample size estimation was calculated using EPI INFO version 6-computer program software.

The prevalence of severe malaria for children admitted in GPW from previous studies was 10%, significance level of 5% (0.05) and a power of 80%.

Therefore the sample size was calculated as follows:

$$N= \frac{\{ z_{\alpha/2}\sqrt{2p(1-p)}+z_{\beta}\sqrt{[p_1(1-p_1)+p_2(1-p_2)]}\}^2}{(p_1-p_2)^2}$$

Where, N = sample size.

$$p= \frac{p_1 + p_2}{2}$$

p_1 and p_2 are the proportions exposed in cases and controls respectively.

p_1 =10%,

p_2 = 35%.

RR (Relative risk) = 3.5.

Z_α = point on standard normal distribution (1.96)

Z_β=1.24

$$N= \frac{\{1.96/2\sqrt{0.45(1-0.225)}+1.24\sqrt{[0.1(1-0.1)+0.35(1-0.35)]}\}^2}{(0.25)^2}$$

N = 48. (Approximately 50 from each group)

Sample size group1 (Severe Malaria children =50)

Sample size group 2 (Uncomplicated Malaria children =50)

Total sample size = 150.

3.6 Study duration

The study was conducted for a period of four months, from July to October 2002 with an average of ten participants in a week.

3.7 Sampling technique and recruitment

Participant recruitment was done only on weekdays. The investigator visited all admitting GPW and recruited all children with severe and uncomplicated malaria as they came after they had met inclusion criteria. On the other hand a list of all children attending Mnazi Mmoja MCH clinic every Tuesday and a list of all children attending OPD at MNH every Friday who met the inclusion criteria were obtained. A simple random technique was used to select candidates to be recruited for the study. Their names were written on small pieces of papers. The papers were then wrapped and mixed up. The investigator randomly picked up ten papers. Every week ten children were recruited for each of the control groups. Enrollment continued up to the time when the total sample size of 150 children was attained. The information and laboratory results were filled in a standardized structured questionnaire designed for the study.

3.8 Data collection

3.8.1 Demographic data

This included: name of the child, address (District), age, sex and use of mosquito nets/INTs.

3.8.2 History/physical examination

The Chief investigator obtained a thorough history and did a physical examination that included symptoms and signs of malaria infection according to WHO criteria.

Body weight was taken using Salter scale or Seca beam balance recorded to the nearest 10 grams.

Temperature was recorded from axilla using mercuric thermometer recorded to the nearest 0.1 degree centigrade.

Palmar pallor was assessed using both hands of the child (compared to the mother or care-taker's palm) to serve as a clinical guideline to severity of anaemia and were recorded as no, some or severe pallor.

3.8.3 Blood slides for malaria parasites

Single sterile pinprick was performed on left index finger after a thorough cleaning of the area with 70% alcohol. A capillary tube was used to draw 0.05 mls of blood and the tube was sealed on one side. This was used for haemoglobin examination.

Three thick blood smears from the same sterile pinprick were prepared. These blood smears were left to dry in air or using Stiebel Electronic machine that took 1 minute to dry. Giemsa stain was applied on the smears and after thirty minutes smears were rinsed using tape water and left again to dry for another ten minutes.

The blood films were examined on an electric microscope under oil immersion using 100x power magnifying lens. A positive malaria slide was recorded as*: Any number of asexual parasites per 200 white blood cells.* The parasite density (the number of asexual parasites per μLitre of blood) was calculated multiplying by a factor 40. A parasite density of ≥10,000 asexual parasites/μL of blood was diagnostic of severe malaria. A negative slide was reported if all three blood slides smears were negative for malaria parasites. To ensure accuracy, a senior laboratory scientist with an experience in malaria research examined all blood slide smears.

3.8.4 Haemoglobin level examination

The sealed capillary tube was centrifuged for five minutes using Centurion – 8000 Series machine at 3000 revolutions per minute.

A Hawksley Micro-Heamatocrit reader machine was used to read and record the Packed Cell Volume (PCV) in percentage. PCV value was converted into haemoglobin levels (g/dl) after dividing it by 3 (a constant Factor).

3.8.5 Plasma Zinc

Inpatients and OPD patients: The anterior cubital area or dorsum of the hand was cleaned thoroughly with 70% alcohol. Using a 5 millilitre disposable plastic syringe with stainless needles, 4 millilitres of venous blood was drawn. Whole blood was put into a plastic vacucontainer with an anticoagulant (EDTA) and mixed thoroughly manually. The specimen were sent into the Specialised Paediatric laboratory (SPL) within thirty minutes where plasma was separated from blood cells by use of Eppendorf Microcentrifuge machine at a 3000 revolutions per minute in order to get plasma. Haemolysed samples were rejected and this necessitated drawing of another sample. Plasma was deep frozen at SPL in Sarstedt micro tubes at temperature -20°C until zinc analysis was done within 2 weeks of blood collection. A *Standard Atomic Absorption Spectrophotometry (SAAS) method for zinc analysis was used. In the SAAS method, zinc atoms absorb light of specific wavelength.*

The absorbed light is directly proportional to the concentration of metal zinc in plasma. The Principal laboratory scientist of Chemistry of the University of Dar es Salaam, performed the zinc analysis on the specimens.

Children from MCH clinic: The Chief investigator and a laboratory technician visited Mnazi Mmoja MCH clinic. Venous blood for plasma zinc determinations was drawn only after meeting inclusion criteria. Four millilitres of venous blood was drawn from the anterior cubital area or the dorsum of the hand and processed as described above. Plasma was immediately deep frozen at a temperature of -20^0 c until the investigator left the clinic for MNH for storing at the SPL. Cold boxes with ice cubes were used to transport deep-frozen plasma from Mnazi Mmoja MCH clinic to MNH and finally to the Chemistry department of the University of Dar es salaam. A plasma zinc level of less than *10.0 μmoles/L* was regarded as zinc deficient.

3.9 Ethical issues/clearance

Parents or guardians were explained thoroughly the aim of this study. Both informed verbal and written consents in either English or Swahili version were signed. Parents or guardian were informed of the usefulness of the study.

Ethical clearance and permission to perform the study was requested and granted by MUCHS high degree ethics on research and publications committee.

3.10 Management and disposal of children

The diagnosed disorders (if any) were communicated to the management team taking care of the child. Patients were managed according to the current management protocols at the centre. At the end of recruitment all children with severe and uncomplicated malaria groups were handed over to the attending Paediatricians for further follow up.

3.11 Quality assurance

During the study period, the following precautions were taken to make sure that there was accuracy and precision.

- It was ensured that only four milliliters of unhaemolysed venous blood was drawn to maintain accuracy.
- Use of free zinc containers, certified by Principle laboratory scientist at Chemistry department of the University of Dar es salaam.
- A two-day training of three research assistants.

  The training of research assistants was focused on proper use of research tools, proper blood collection and storage.
- Proper data entry into the computer with assistance from an experienced computer program and data analysis.

3.12 Data Analysis

Data analysis was done using EPI INFO- version 6 program. The chief Investigator carried out data entry with some help from a computer program officer familiar with research activities. Statistical significant tests by odds ratio (OR), where indicated were calculated using a two-tailed p-value (Fishers). Mean ages were analyzed using analysis of variances (ANOVA). A p -value of < 0.05 was considered statistically significant.

3.13 Limitations of the study

Haemolysed blood samples were unsuitable because red blood cells contain 80% of total body zinc. This necessitated repeat of the procedure in drawing another venous blood or recruitment of new subjects, hence more resources were used. There were few children without malaria above two years of age who attended routine growth monitoring and immunization at Mnazi Mmoja MCH clinic.

4.0 RESULTS:

A total of 2074 children were admitted in the general paediatric wards of the Muhimbili National Hospital during the study period (July to October 2002). All 100 mothers or caretakers of children with severe or uncomplicated malaria consented for inclusion into the study.

Three mothers from Mnazi Mmoja MCH clinic refused to sign for their children to participate and were excluded from the study. Eleven-blood samples haemolysed that necessitated for recruitment for new subjects or repeated venous blood withdraw.

Table1: Population distribution of children on malaria severity according to age and sex.

	Severe malaria n (%)	Uncompl malaria n (%)	No malaria n (%)	Total n (%)	p-value
Sex Male	32 (64)	27 (54)	26 (52)	85 (56.6)	+ 0.673
Female	18 (36)	23 (46)	24 (48)	65 (43.3)	* 0.731
Age (mo)					
1-24	29 (58)	39 (78)	43 (86)	111 (74)	+ 0.159
25-59	21 (42)	11 (22)	7 (14)	39 (26)	* 0.081
Mean Age (±SD)	23.480 (±16.853)	18.280 (±15.880)	13.260 (±11.412)		

Note: Statistical analysis.

+ Indicate, severe malaria Vs uncomplicated malaria children.

* Indicate, severe malaria Vs no malaria children.

A total of one hundred and fifty children (56.6 % males and 43.4% females) aged 1 to 59 months were enrolled for the study. Thirty-two (64%) from severe, twenty-seven (54%) from uncomplicated and twenty-six (52%) from no malaria children groups were males.

Twenty-nine (58%) from severe malaria, thirty-nine (78%) from uncomplicated and forty-three (86%) from no malaria groups were ≤ 24 months of age.

There were no statistical significance differences on population distribution on age groups and sex with a p- value of > 0.05 Table 1.

Table 2: Association between plasma Zinc status and malaria severity.

Plasma Zinc	Severe Malaria n (%)	Uncompl Malaria n (%)	No Malaria n (%)	Total n (%)	Odds ratio & 95%CI	P-value
Low	42 (84)	29 (58)	28 (56)	99 (66)	+ 3.8 (1.5,10)	+ 0.007
Normal	8 (16)	21 (42)	22 (44)	51 (34)	* 4.1 (1.6,11)	* 0 .004
Mean (±SD)	7.019 (±4.052)	11.031 (±5.43)	12.490 (±8.31)			

Note: Statistical analysis.

+ Indicate, severe malaria Vs uncomplicated malaria.

* Indicate, severe malaria Vs no malaria children

In the studied groups;

Forty-two (84%) of all severe malaria, twenty-nine (58%) of all uncomplicated malaria and twenty-eight (56%) of all children without malaria had low plasma zinc levels.

There was a significant association on low plasma zinc levels and malaria severity in all studied groups. The differences between groups were statistically significant.

The odds ratio on low plasma zinc levels between severe malaria and uncomplicated malaria was 3.8 (95% CI 1.5,10) with a p-value of 0.007.

The odds ratio on low plasma zinc levels between severe malaria and children without malaria was 4.1(95% CI 1.6,110) with a p value of 0.004. The mean plasma zinc levels for severe malaria was 7.019 μmol/L (±4.052) while 11.031 μmol/L (±5.43) for uncomplicated and 12.490 μmol/L (±8.31) for children without malaria groups.

Table 3: Association between Plasma Zinc levels and malaria severity by age groups.

Plasma Zinc	Age (mo)	Severe Malaria n (%)	Uncompl Malaria n (%)	No Malaria n (%)	Total n (%)	Odds ratio & 95%CI	P-value
Low	≤ 24	23 (55)	22 (76)	22 (79)	67 (68)	+0.39 (0.2,1)	+0.129
	>24	19 (45)	7 (24)	6 (21)	32 (32)	*0.3 (0.1,1)	*0.105
Normal	≤24	6 (75)	17 (81)	21 (95)	44 (86)	+1.4 (0.2,9)	+0.147
	>24	2 (25)	4 (19)	1 (5)	7 (14)	* 7 (0.5,11)	* 0.43

Note: Statistical analysis.

+ Indicate, severe malaria Vs uncomplicated malaria children

* Indicate, severe malaria Vs no malaria children

There were two age groups for the purpose of this study. The first groups were children ≤ 24 months of age. The second group were children > 24 months of age.

One hundred and eleven (74%) of all children studied were below or equal to 24 months of age. Sixty-seven (68%) of all children below or equal 24 months of age and thirty-two (32%) of all children above 24 months of age had low plasma levels.

Twenty-three (55%) of all children aged ≤ 24 months with severe malaria and twenty-two (76%) of all children aged ≤ 24 months with uncomplicated had low plasma zinc levels. Twenty-two (79%) of all children aged ≤ 24 months without malaria had low plasma zinc levels. The differences were not statistically significant on low plasma zinc levels and malaria severity by age groups. The OR for low plasma zinc levels between severe malaria and uncomplicated malaria for children ≤ 24 months age groups was 0.39 (95% CI 0.2, 1) with a p- value of 0.129. The OR for low plasma zinc levels between severe malaria and without malaria children ≤ 24 months age groups was 0.3 (95% CI 0.1,1) with a p-value of 0.105.

Table 4: Association between Plasma Zinc levels and malaria severity by sex groups.

Plasma Zinc	Sex	Severe Malaria n (%)	Uncompl Malaria n (%)	No Malaria n (%)	Total n (%)	Odds ratio & 95% CI	p-value
Low	Male	28 (56)	18 (36)	16 (32)	62 (41)	+1.2 (0.5,3)	+ 0.801
	Female	14 (28)	11 (22)	12 (24)	37 (25)	*1.5 (0.6,4)	* 0.548
Normal	Male	4 (8)	9 (18)	10 (20)	23 (15)	* 0.83 (0.2,4)	* 1.000
	Female	4 (8)	12 (24)	12 (24)	28 (19)	+ 0.75 (0.2,4)	+ 0.135

Note: Statistical analysis.

+ Indicate, severe malaria Vs uncomplicated malaria children.

* Indicate, severe malaria Vs no malaria children.

There were eighty-five males (56.7%) in the studied population. Sixty-two (73 %) of all males and thirty-seven (56.9%) of all females had low plasma zinc levels. Twenty-eight (56%) of all males from severe malaria, eighteen of all males (36%) from uncomplicated and sixteen of all males (32%) from children without malaria groups had plasma low zinc levels. There were more males in all three groups of children studied who had lower plasma zinc levels than females but the differences were not statistically significant.

The OR for low plasma zinc levels between severe malaria and uncomplicated malaria on sex groups was 1.2 (95% CI 0.5, 3.3) with a p-value of 0.801. The OR for low plasma zinc levels between severe malaria and without malaria children groups on sex groups was 1.5 (95% CI 0.6, 4) with a p-value of 0.548. Table 4.

Table 5: Association between plasma zinc levels and malaria severity by use of insecticide treated bed nets (ITNs).

Plasma Zinc	Severe Malaria n (%)	Uncompl Malaria n (%)	No Malaria n (%)	Total n (%)	Odds ratio & 95%CI	p-value
Normal	1 (9)	11 (61)	12 (46)	24 (44)	+ 0.1 (0.01,0.6)	+ 0.002
Low	10 (91)	7 (39)	14 (54)	31 (56)	* 0.12 (0.01,1)	* 0.005
Total	11	18	26	55		

Note: Statistical analysis.

+ Indicate, severe malaria Vs uncomplicated malaria children.

* Indicate, Severe malaria Vs no malaria children.

Fifty-five (39%) of all mosquito net users reported use of insecticide treated mosquito bed nets (ITNs). Twenty-six (47.3%) children without malaria, eighteen (32.7%) from uncomplicated and eleven (20%) from severe malaria groups used ITNs.

One (9%) from severe, eleven (61%) from uncomplicated and twelve (46%) without malaria group children who used ITNs had normal plasma levels. The differences for low plasma zinc levels and malaria severity on use of ITNs were statistically significant among groups studied.

The OR of low plasma zinc levels among users of ITNs between severe malaria and uncomplicated malaria was 0.1 (95% CI 0.01, 0.6) with a p-value of 0.002. The OR of low plasma zinc levels among users of ITNs between severe malaria and without malaria children was 0.12 (95% CI 0.01,1) with a p-value of 0.005. Low OR here meant that ITNs were protective by 90% and 88% respectively among the two groups as seen in Table 5.

Table 6: Association between plasma zinc levels and malaria severity on nutritional status by WFA.

Plasma Zinc	WFA	Severe Malaria (%)	Uncompl Malaria n (%)	Total n (%)	Odds ratio & 95% CI	p-value
Low	<80	15 (30)	10 (20)	25 (25)		
	≥80	27 (54)	19 (38)	46 (46)	+1.1 (0.4,2.9)	+1.000
Normal	<80	5 (10)	4 (8)	9 (9)		
	≥80	3 (6)	17 (34)	20 (20)	+7.1 (7.1,43)	+0.667

Note: Statistical analysis.

+ Indicate, severe malaria Vs uncomplicated malaria children.

Using Tanzania MCH card number one, a child whose weight for age < 80% he/she was considered as malnourished while a child's weight for age ≥ 80% he/she was considered as normal.

Thirty-four (34%) out of all 100 children with malaria had malnutrition. Twenty-five (73.5%) out of all 34 malnourished children had low plasma zinc levels. Malnourished children with low plasma zinc levels were further stratified. Severe malaria children who were malnourished with low plasma zinc levels were fifteen (30%) while children who had uncomplicated malaria and malnourished were ten (20%). However there was no statistical significant difference on low plasma zinc levels and malaria severity regarding nutritional status using WFA. The OR for low plasma levels between severe malaria and uncomplicated malaria regarding nutritional status was 1.1 (95% CI 0.4, 2.9) with a p-value of 1.000.

5.0 DISCUSSION.

This study found significant differences in plasma zinc levels among groups of children studied. This difference shows a progressive decrease in plasma zinc levels as malaria severity increased. This might indicate that as a child progress from no malaria to uncomplicated and severe malaria states, plasma zinc levels continue decreasing. This progressive decreasing plasma zinc levels lead us to speculate that not only plasma zinc are associated with severe malaria, but direct to us a causal pathway.

There were 4.1 times risk of low plasma zinc levels among children with severe malaria compared to those who had no malaria. While the risk of low plasma zinc levels among uncomplicated malaria and severe malaria was 3.8. Therefore the estimated risk of low plasma zinc levels among severe malaria and those with uncomplicated malaria was almost 4.

Fifty six percent of children without malaria who were less than five years of age and attending a routine MCH clinic were zinc deficient. In one study it indicated that 38-50% of all children in developing countries are zinc deficient.[23] Our study population revealed that normal or healthy children had a high prevalence of low plasma zinc levels than evidenced from other developing countries.

The reasons for above findings could by the fact that, children in this part of the country experience 4-6 attacks of malaria in a year and so constantly exposed to low zinc levels before subsequent attacks.[1] The staple food, even during weaning period is mainly cereal-based type with phytate, which forms an insoluble complex with zinc and makes it unable to be absorbed in the proximal intestines.[20, 24] It is also evident that febrile illnesses have got a negative effect on plasma zinc more so during acute phase reaction (APR) as it is for malaria infection. [21]

This case control study has been able to shed some light on the relation of plasma zinc levels in children less than five years of age with and those without malaria. Most previous studies were prospective studies on malaria case management with randomized trials of oral zinc supplementation. These studies concluded that oral zinc supplementation in malaria endemic areas reduced frequencies of health centre attendance and heavy parasitemia due to P. falciparum malaria.[27-34] Although it may be implied that zinc is useful in outcomes of malaria; a causal pathway has not been elucidated. However whether low plasma zinc levels predispose to severity of malaria or whether severe malaria further depletes the already low levels of zinc remain unknown.

This trend of serial decline in plasma zinc levels may indicate an early warning sign of impending severe malaria attack. This might signify that prompt efforts should be put into effect to prevent these children from progressing to severe forms of plasmodium infection. The magnitude of the problem worsened from 56% from children without malaria to 58 % and 84 % from uncomplicated and severe malaria respectively. In this study the mean plasma zinc levels declined from 12.49 μmols/L (± 8.31) for children without malaria to 11.031 μmols/L (±5.43) and 7.019 μmols/L (± 4.052) for uncomplicated and severe malaria children respectively, further explaining above scenario.

Hocke et al concluded that inadequate zinc intake were highly prevalent in developing countries especially during the period of complementary feeding when zinc requirement are high and breast milk contribute little.[19] This study tend to agree with that information as low plasma zinc levels was more prevalent in children ≤ 24 months than children > 24 months of age in each of the studied groups. However there were no statistical significance differences on low plasma zinc levels and malaria severity in terms of age groups. The findings could be explained by unequal population distributions. There were 111 children ≤ 24 months against 39 children > 24 months of age.

There were seven children (14%) > 24 months of age from no malaria group who were recruited at the routine MCH clinic. This indicated that as a child completes the Tanzania primary immunizations (according to the Expanded program on immunization – EPI) after age of one year, frequency and number of attendances declined. This study was an unmatched case control; hence a matched type could probably explain the differences in terms of age groups. Both two age groups studied, that was probably the period where there were more repeated parasitic infections such as malaria, pneumonia and diarrhoea diseases.

Malnutrition further might be responsible for low plasma zinc levels in children in both two age groups. This study did not go into the details of breast-feeding whether exclusive, the duration and type of complementary food. However majority of children in this part of the country are breast-fed up to the age of two years, and complementary feeding indicate a zinc deficient diet. King et al noted a male predominance as far as susceptibility to plasma zinc deficiency but they responded more readily to zinc supplementation. [16] In this study generally there were more males than females and the male female ratio was 2:1.However there was no statistical significant difference on low plasma zinc levels and malaria severity on sex groups.

There aren't any clear scientific explanations at the moment to explain as to why males are more susceptible to zinc deficiency. It may be due to genetic composition and hormonal functions. Likewise, it is not known whether males respond readily to zinc supplementation. This gender difference and zinc levels described by King et al need further exploration.

There was a high rate of mosquito net use, ninety four percent of the population studied reported sleeping under mosquito nets at night. This was a good response but there could be some information bias, as the investigators had no means of verifying these reports. There aren't studies to indicate a direct relationship between mosquito nets use (plain or ITNs) and plasma zinc levels. However other studies had concluded that a mosquito net lowers the incidence of malaria attacks and possibly severity.[45] In that context it could have a positive effect on plasma zinc levels indirectly. Fifty percent of children without malaria group, who had used ITNs, had normal plasma zinc levels as compared to uncomplicated and severe malaria groups (45.5% and 42% respectively). The differences on low plasma zinc levels and malaria on use of ITNs were statistically significant.

The risk (OR) for using ITNs and getting low plasma zinc levels from uncomplicated malaria was 0.1 while that from no malaria group was 0.12. It should be clear that for ITNs users, the lower the OR the higher the protection against severe malaria with increased chances for normal plasma zinc levels. Ninety percent of ITNs users had normal plasma zinc levels. However this finding does not indicate that ITNs per se maintains normal plasma zinc levels. There might be some indirect effects. ITNs have mainly two roles on malaria control. ITNs act as mosquito bite barriers and also as mosquito killers giving synergistic effects, thereby decreasing the incidence of malarial infection.

Malnourished children with severe malaria and low plasma zinc levels were greater. Fifteen (30%) malnourished children from severe malaria and ten (20%) from uncomplicated malaria groups had low plasma zinc levels. However, the results on low plasma zinc levels and malaria severity among malnourished children were not statistical significant. The risk ratio for low plasma zinc levels and nutritional status was 1.1.

In a study done at MNH, 74% of all malnourished children admitted at the nutrition centre had zinc deficiency.[22] However the selection criteria used in the MNH study differed from our study. The selected criterion (WFA) in our study measured mainly if the child had protein deficiency.

Low plasma zinc is commonly encountered in children who are protein deficient and that albumin is a zinc carrier. It is known that children who are protein deficient are also albumin deficient [21]. The MNH study included children with underweight, stunting, wasting, and other forms of severe malnultrion (kwashiorkor, marasmus and marasmic-kwashiokor). [22] It has been noted that zinc supplementation in children with significant growth delay resulted in increased growth rates compared to placebo.[18] Whether low plasma zinc predisposes these children to get malnutrition and malaria or whether malaria predisposes them to get malnutrition and low plasma zinc levels cannot be answered directly by this study.

This can be explained from immunology perspectives that children with malaria are also prone to get other infections that can lead to malnutrition. On the other hand malnourished children also are prone to get other infections that can lead to low plasma zinc levels and increase malaria severity.

6.0 CONCLUSIONS

- Low plasma zinc levels were significantly associated with malaria severity in children less than five years of age in GPW, MNH. Eighty four percent of severe malaria had low plasma zinc levels. Fifty eight percent of uncomplicated malaria had low plasma zinc levels with an estimated relative risk (OR) of 3.8 developing severe malaria.
- More than a half (i.e.56%) of all children less than five years of age without malaria attending MHC clinic had low plasma zinc levels with an estimated relative risk (OR) of 4.1 developing severe malaria.
- ITNs use in children less than five years of age from Dar es Salaam had a protective effect of 90% against severe malaria with increased plasma zinc levels.
- Age, sex and nutritional status in children less than five years of age were not risk factors for low plasma zinc levels and malaria severity.

7.0 RECOMMENDATION

- There is a need for large scale studies (prospective cohort) to elucidate the causal relationship of zinc and malarial infection and recommend public health measures for zinc supplements.

8.0 REFERENCES

1. WHO, CDC: Severe Falciparum malaria. Trans R Soc Trop Med Hyg 2000; 94 (Suppl 1):1-90.
2. Ramasamy R. Molecular basis for evasion of host immunity and pathogenesis in malaria. Biochimica et Biophysica Acta 1406 (1998): 10-27.
3. Snow RW, Marsh K. New Insights into the epidemiology of Malaria relevant for disease control. Br.Med.Bull.54 2 (1998) 293- 309.
4. Desowitz RS. Statistics on Malaria Control Programme, WHO 3rd World Network features, CDC. W.W Norton & Company, NewYork 1999.
5. Newton CR, Krishna S. Severe falciparum in children: Current understanding of pathophysiology and supportive treatment. Pharmacology and Therapeutics 79 (1998): 1-53.
6. Mwaluko GMP, Kilama WL, Mandara MP et al. Health & Diseases in Tanzania. Herper Collins Academic 1991:117-29.
7. Msengi A. Malaria control Past , Present and New Opportunity for the next millennium: The 1999 Michael Ngwiramungu memorial lecture. TMJ 2000, 15 (1): 1-8.
8. Fataki MR, Manji KP, Premji Z: Rising trend of malaria morbidity and mortality in children at MNH. TMJ June 2002, 17(2): 11-15.

9. Sharma YD. " Knob proteins in falciparum malaria. Indian Journal of medical Research 106 (1997): 53-60.

10. Hyder AA, Rotlant G, Morrow RH. Measuring the burden of disease: Health life years. Am J Public Health. 1998; 88 (2): 196-202.

11. Beier JC. Malaria parasite development in mosquitoes. Ann Rev Entomol.1998; 43:519-543.

12 World Health Organization. Malaria prevention and control. Online WHO/CTD. www.who.int/ctd/html/malaria html.25th Febr.1999.

13. Cullinan TR, Pieterick CF. Packaged treatment for first-line care in cerebral malaria and meningitis. World Health Organisation Bulletin 76 (1998): 257-264.

14. National guidelines for malaria diagnosis and treatment - United Republic of Tanzania MOH; Malaria Control series 2000 (1): 9-22.

15.UNICEF report; Panel18. Zinc and vitamin A. Taking the sting out of malaria. The state of world's children 1998.

16. King JC, Keen CL. Zinc.In: Shils M, Oslon JA, Shike M, Ross AC, eds. Nutrition in Health and Diseases. 9th Eds, Baltimore: Williams & Wilkins, 1999:223-239.

17. O' Brien K O, Zavaleta N, Caulfield LE, et al. Prenatal iron supplements impairs zinc absorption in pregnancy Peruvian women. Journal of Nutrition 2000; 130: 2251-2255.

18. Hocke M, Winnefeld K, Bosseckert H, et al. Oral Zinc therapy in patients with supposed mild Zinc deficiency. Z. Gastroenterol 2001 39 (1): 83-88.

19. William FG. Energy balance, metabolism and nutrition. Mineral requirements. Review of medical Physiology 14th eds. Appleton & Lange medical book 1989: 262- 264.

20. Solomon NW, Cousin RJ. Zinc absorption and malabsorption of mineral nutrients (eds) Alan R Liss 1984: 125-197.

21. Trace element zinc (Zn). Joint venture of London health services. London laboratory service Group. www.lhsc.on.ca/lab/metallab/elements/30zn.htm. 15th November 2002.

22. Mushi SM. The effect of Zinc supplementation in children recovering from malnutrition at MMC; Dissertation submitted in Partial fulfilment. MMed University of Dar es Salaam 1997.

23. Rajiv B, Nita B, Michael HK, et al. Plasma zinc as a predictor of Diarrhoea and Respiratory morbidity in children in an urban slum setting. Am J Clin Nutr 1998; 68 (suppl); 414s- 417s.

24. Institute of Medicine. Dietary reference intakes for vitamin A, vitamin K, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc. Washington, DC: National Academy Press. 2001:1214-7.

25.Caulfield LE, Zavaleta N, Shankar AH, et al. Potential contribution of maternal zinc supplementation during pregnancy to maternal and child survival. Am J Clin Nutr.1998; 68 (2 suppl):4995-5085.

26. Krebs NF. Zinc supplementation during lactation. Am J Clin Nutr 1998; 68 (2 suppl): 509s-512s.

27. Black RE. Therapeutic and preventive effects of zinc on serious Childhood infectious diseases in developing countries. Am J Clin Nutr 1998: 68 (2 suppl): 476s-479s.

28. Shankar AH, Genton B, Tamja S, et al. Zinc supplementation can reduce Malaria -related morbidity in pre-school children. Am J Trop Med Hyg 1997; 57: A 434.

29. Shankar AH, Genton B, Baisor M, et al .The influence of zinc supplemantation on morbidity due to plasmodium falciparum: a randomised trial in preschool children in Papua New Guinea. Am J Trop Med Hyg. 2000 Jun;62(6):663-9.

30. Shankar AH, Kumar N, Scott AL. Zinc deficiency exacerbates experimental malaria infection in mice. FASEB J 1995;9:A4269.

31. Bates CJ, Evans PH, Dardenne M, et al. A trial of zinc supplementation in young rural Gambian children. Brit. J Nutr.1993; 69:243-5.

32. Nussenblatt V, Semba RD. Micronutrient malnutrition and pathogenesis of malarial anaemia. Acta Trop 2002; 82(3):321-37.

33. Black RE, Sazawal S. Zinc and childhood infectious disease morbidity and mortality. Br. J Nutr 2001; 85 (2 Suppl): S125-9.

34. Brown KH. Effect of Infections on plasma zinc concentration and implications for zinc status assessment in low-income countries. Am J Nutr 1998 Aug; 68 (2 suppl): 435s-441s.

35. Christian P, West KP Jr. Interactions between zinc and Vitamin A. an update. Am J of Clin Nutr. 1998; 68 (2suppl): 4705- 4755.

36. Hambridge KM. Zinc and diarrhoea diseases in young children. Acta Paediatr 1992; suppl 381:82-6.

37. Moynahan EJ. Acrodermatitis enteropathica: a lethal inherited human zinc deficiency disorder. Lancet.1974; 17:2(7877): 399 - 400.

38. Prasad AS. Zinc deficiencies in women infants and children. J Am Coll Nutr. 1996;5(2);113-20.

39. Bhutta ZA, Black RE, Brown KH, et al. Prevention of diarrhoea and pneumonia by zinc supplementation in children in developing countries; pooled analysis of randomised controlled trials. Zinc Investigators' Collaborative Group. J of Pediatr.1999;35(6):689-697.

40.Walravens PA, Hambidge KM, Koepfer DM. Zinc supplementation in infants with nutrition pattern of failure to thrive. a double blind controlled study. Pediatr.1989:83: 532-538.

41. MacDonald RS. The role of Zinc in growth and cell proliferation. J Nutr.2000; 130:1500s-15008s.

42. Black MM. Zinc deficiency and child development. Am J Clin Nutr.1998; 68(2 suppl): 464s-469s.

43. Chuwa LM, Mwiruki G, Bilal MG, et al. Serum iron, zinc, copper and bromide in malnourished children in Dar es Salaam, Tanzania. EAMJ 1996; 73 (suppl); 521-3.

44. Zinc Against Plasmodium Group. Effect of zinc on treatment of Plasmodium falciparum malaria in children: A randomised controlled trial. Am J Clin Nutr. 2002; 76 (4): 805-12.

45.Malaria and bed nets: Fact sheet. www.idrc.ca/books/reports/1996/01.06e.htm 24th January 2003.