

Serum Vitamin A and Zinc levels in School Children with and without Malaria Parasitaemia in Kisii District, Kenya.

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A thesis submitted in partial fulfillment for the Degree of Master of Science in Public Health, in the Jomo Kenyatta University of Agriculture and Technology.

2010

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

This thesis is dedicated to my children; Duncan Kerongo, Briane Bosire, Laura Kerubo and Tracy Moraa.

Love what you want to do. This is tough but you must learn to love the practice, the sweat, the trials ---- in a word – everything.

If you really want to succeed in this competitive world, you have to love a challenge.

Learn to appreciate setbacks as necessary growth.

Always believing in your heart that your hard work will be rewarded.

ACKNOWLEDGEMENTS

My first gratitude goes to my supervisors: Mr. Charles F.L Mbakaya, Dr. Yeri Kombe and Dr. Venny Nyambati for the guidance, patience, time and dedication accorded to me.

I acknowledge the management of Kisii District Hospital for allowing me to use their facilities and Kisii District Hospital staff especially Matron Agnes Monayo and members of Vector Control Unit; Mr. Karanja, Mr. Maobe, and Mr. Mayaka.

I appreciate the help I received from the administration, teachers and parents/guardians of the seven primary schools namely; Mosache, Nduru, Mugori, Mwata, Genga, Nyandiwa and Metembe. They acknowledged the need for my study and gave me consent to work with their children. To all the pupils who participated, my greatest gratitude.

Special thanks to S. Momanyi, L. Njogu, C. Kagendo, P. Obaigwa, D. Wattimah, F. Mogotu, A. Abuga, D. Kerongo and L. Kerubo for helping me piece the data together. I take this opportunity to thank Moses Mwangi of CPHR/KEMRI for assisting me during data analysis.

I will forever be grateful to everyone else who played a part in this study.

Last but not least I thank my children for loving me and being there for me, and my mother for her moral support and great counsel.

TABLE OF CONTENTS

DECLARATION	iii
DEDICATION	iv
ACKNOWLEDGEMENTS	v
TABLE OF CONTENTS.....	vi
LIST OF TABLES.....	xi
LIST OF FIGURES	xii
LIST OF APPENDICES.....	xiii
LIST OF ABBREVIATIONS	xiv
ABSTRACT	xvi
CHAPTER ONE: INTRODUCTION	1
1.1 Background.....	1
1.2 Statement of the Problem	3
1.3 Justification for the Study	4
1.4 Null Hypothesis	4
1.5 Objectives.....	4
1.5.1 General Objectives.....	4
1.5.2 Specific Objectives.....	5
CHAPTER TWO: LITERATURE REVIEW	6
2.1 Malaria Transmission.....	6
2.2 The devastating impact of Malaria	8
2.3 Zinc	11

2.3.1 Role of zinc.....	11
2.3.2 Bioavailability of zinc	12
2.3.3 Distribution of zinc in the body	13
2.3.4 Requirements of zinc in children	13
2.3.5 Zinc deficiency	14
2.3.6 Zinc toxicity.....	14
2.3.7 Zinc and immunity	15
2.4 Vitamin A	16
2.4.1 Nature and sources of Vitamin A.....	16
2.4.2 Vitamin A bioavailability	17
2.4.3 Absorption, transport and storage of vitamin A	17
2.4.4 Recommended Intakes	18
2.4.5 Vitamin A deficiency	19
2.4.6 Toxicity of vitamin A.....	19
2.4.7 Functions of vitamin A.....	20
2.5 Malaria and Iron status.....	23
CHAPTER THREE: MATERIALS AND METHODS	26
3.1 Study Design	26
3.2 Study Site	26
3.3 Study Population.....	28
3.3.1 Inclusion Criteria	28
3.3.2 Exclusion Criteria	28

3.4 Determination of Sample Size	29
3.5 Sampling Procedure	30
3.6 Data Collection	31
3.6.1 Study Questionnaire	31
3.6.2 Food Intake Frequency	32
3.6.3 Clinical signs	32
3.6.3.1 Heart/pulse rate	32
3.6.3.2 Respiratory rate	33
3.6.3.3 Blood pressure.....	33
3.6.3.4 Temperature	33
3.6.4 Nutrition status.....	34
3.6.4.1 Anthropometric measurements	34
3.6.4.2 Body Mass Index (BMI).....	35
3.6.4.3 Mid Upper Arm Circumference (MUAC).....	35
3.6.5 Blood collection and analysis	35
3.6.5.1 Diagnosis of malaria.....	35
3.6.5.2 Blood collection	36
3.6.5.3 Full Haemogram	36
3.6.5.4 Vitamin A analysis	36
3.8.5 Zinc analysis	37
3.7 Data management and analysis.....	38
3.8 Ethical Considerations	39

CHAPTER FOUR: RESULTS	40
4.1 Socio-demographic characteristics	40
4.1.1 Gender and age of study population.....	40
4.1.2 Guardian/parent occupation.....	40
4.1.3 Guardian/parent level of education	41
4.1.4 Reported morbidity among the children in the past one month.....	41
4.1.5 Use of mosquito control methods by study population.....	43
4.2 Food Intake Frequency.....	43
4.3 Clinical Signs.....	44
4.3.1 Temperature.....	44
4.3.2 Respiratory rate.....	45
4.3.3 Pulse Rate	45
4.3.4 Blood pressure	46
4.4 Nutrition Status.....	47
4.4.1 Mid Upper Arm Circumference (MUAC).....	47
4.4.2 Body Mass Index (BMI).....	49
4.4.3 Nutrition Z scores by malaria status.....	49
4.5 Micronutrient status	50
4.5.1 Haematology profile.....	50
4.5.2 Retinol status	53
4.5.3 Zinc status.....	55
4.5.4 Serum retinol and zinc levels.....	56

CHAPTER FIVE: DISCUSSION	59
5.1 Socio-demographic characteristics and malaria status.....	59
5.2 Nutritional status of the children and malaria status.....	60
5.3 Frequency of feeding and malaria status.....	60
5.4 Clinical signs and malaria status.....	62
5.5 Haemogram profile and malaria status	63
5.6 Serum zinc levels and malaria status	65
5.7 Serum vitamin A and malaria status	66
5.8 Study Limitations.....	68
CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS	69
6.1 Conclusions	69
6.2 Recommendations.....	70
REFERENCES	71
APPENDICES	84

LIST OF TABLES

Table 1:	Distribution of study population by school and District.....	31
Table 2:	Guardian/Parent occupation.....	40
Table 3:	Highest level of parent/Guardian education	41
Table 4:	Demographic and health related factors by Malaria status of the children	42
Table 5:	Distributrition of Malaria by use of mosquito control among study population	43
Table 6:	Frequency of feeding per week by Malaria status.....	43
Table 7:	Children's temperature (°c) by Malaria status.....	44
Table 8:	Pulse rate (Beats/Min) by Malaria status.....	46
Table 9:	Clinical profile by Malaria status	47
Table 10:	MUAC values by Malaria status	48
Table 11:	BMI values by Malaria status	49
Table 12:	Children Z scores by Malaria status	50
Table 13:	Haemoglobin levels by Malaria status.....	51
Table 14:	Full Haemogram by Malaria status	50
Table 15:	Association between serum retinol levels and Malaria	54
Table 16:	Mean serum zinc legel ($\mu\text{g} / \text{dL}$) by Malaria status.....	55

LIST OF FIGURES

Figure 1:	The life cycle of malaria	7
Figure 2:	Respiratory rate (respirations/min) by Malaria status	45
Figure 3:	Blood pressure (mm/Hg) by malaria status	46
Figure 4:	Mid Upper Arm Circumference (cm) by malaria status	48
Figure 5:	Retinol level ($\mu\text{mol} / \text{L}$) by malaria status	54
Figure 6:	Serum zinc level ($\mu\text{g} / \text{dL}$) by malaria status	56
Figure7:	A scatter plot showing the relationship between retinol and zinc	57
Figure 8:	A scatter plot showing the relationship between retinol and zinc by gender	58

LIST OF APPENDICES

Appendix 1: Kemri-ERC Ethical Approval copy.....	83
Appendix 2: Study Information Sheet	84
Appendix 3: Study Consent Form	86
Appendix 4: Vitamin A and Zinc Study Questionnaire.....	87
Appendix 5: Food Frequency Questionnaire	90

LIST OF ABBREVIATIONS

ANOVA:	Analysis of Variance
BMI:	Body Mass Index
BMR:	Basal Metabolic Rate
BP:	Blood Pressure
DD:	Dietary Diversity
CPHR:	Centre for Public Health and Research
DNA:	Deoxy-ribonucleic acid
FAAS:	Flame Atomic Absorption Spectrometry
FAO:	Food and Agriculture Organization
FFQ:	Food Frequency Questionnaire
FNB:	Food and Nutrition Board
HAZ:	Height-for-Age
Hb:	Haemoglobin
HPLC:	High Performance Liquid Chromatography
IAEA:	International Atomic Energy Association
INACG:	International Anaemia Consultative Group
IOM:	Institute of Medicine
ITROMID:	Institute of Tropical Medicine and Infectious Diseases
I.U:	International Units
JKUAT:	Jomo Kenyatta University of Agriculture and Technology
KEMRI:	Kenya Medical Research Institute

MCH:	Mean Corpuscular Hemoglobin
MCHC:	Mean Corpuscular Hemoglobin Concentration
MCV:	Mean Corpuscular Volume
MOH:	Ministry of Health
MUAC:	Mid Upper Arm Circumference
NRC:	National Research Center
NCHS:	National Centers for Health Statistics
NHANES:	National Health and Nutrition Examination Survey
PCV:	Packed Cell Volume
PEM:	Protein Energy Malnutrition
RBC:	Red Blood Cells
RBP:	Retinol-binding protein
RDA:	Recommended Daily Allowance
RE:	Retinol Equivalent
RNA:	Ribonucleic acid
RNI:	Recommended Nutrient Intake
SI:	International System (of units of measurement)
UNICEF:	United Nations Children's Fund
WAZ:	Weight-for- Age
WBC:	White Blood Cells
WHO:	World Health Organization
WHZ:	Weight-for-Height

ABSTRACT

Zinc and vitamin A supplementation in children has been associated with reduction in the incidence and severity of diarrheal diseases, acute respiratory infections and malaria. However, studies have shown little evidence of zinc supplementation on reduction of morbidity and malaria. The aim of this study was to identify the possible association between serum retinol and zinc levels and susceptibility to malaria infection in school children. This was a case-control study. A finger prick was done to obtain blood from school children aged between 6 – 10 years to screen for malaria. In total, 55 children with malaria parasitaemia and a similar number without were enrolled in the study. Venous blood was drawn from the children and analyzed for serum levels of zinc retinol and haemoglobin. Atomic Absorption Spectrometry, and High performance liquid chromatography [HPLC] were used in determination of serum zinc and retinol respectively. Anthropometric measurements and clinical signs of malaria were taken. A questionnaire on demographic data, dietary practices, use of anti-vector measures and relevant medical history was administered on the children's parents/guardians. Data was cleaned and entered into a database using SPSS version 11.5. Epi Info v 6.04 was used to classify anthropometric indices. Descriptive statistics such as mean, standard deviation, median and range were used to indicate the average value and spread of these values. Independent T-test was used to test for mean comparison on the continuous variables between malaria positive and malaria negative children. Pearson's correlation coefficient was used to measure relationships between two continuous variables. Cross tabulations between all categorical variables and malaria status was

performed to uncover the distribution patterns. Pearson chi-square test was used to test for association between malaria status and individual categorical variables.

Children with and those without malaria had similar serum zinc ($P=0.762$) and retinol ($P=0.402$) values although those with malaria had slightly higher levels of both serum zinc and retinol. However, children without malaria had higher mean blood count (304.8) than those with malaria (257.6), significant at $P=0.001$. Although not statistically significant, children without malaria had higher mean iron levels than children with malaria. The study confirms other findings that the link between serum zinc and retinol and malaria is not clear. Interestingly, children without malaria had significantly higher frequencies of feeding per week compared to those with malaria. More in depth investigations are needed to conclusively establish the relationship between serum zinc and retinol levels and malaria parasitaemia.

CHAPTER ONE

INTRODUCTION

1.1 Background

Malaria is a life threatening parasitic disease transmitted by mosquitoes. It is caused by a protozoa parasite called *Plasmodium*, which has four species that infect humans- *Plasmodium vivax*, *P. malariae*, *P. ovale*, and *P. falciparum*. All four species of human *Plasmodium* occur in Kenya. *P. falciparum*, which causes the severest form of the disease, accounts for 98 per cent of all malaria infections. The major malaria vectors in Kenya are members of the *Anopheles gambiae* complex and *An. Funestus* (Abraham and James, 1995).

The government of Kenya recognizes malaria as a health and social-economic burden and considers malaria control a priority investment necessary for the realization of Kenya Vision 2030. Malaria is responsible for 30 % of outpatient consultations, 19% of hospital admissions and 3-5 % of inpatient deaths. 70 % of Kenyan population lives in malarious areas. In 2007, there were 9.2 million reported clinically diagnosed malaria cases in the public health sector (HMIS, 2008).

Kenya has four malaria epidemiological zones based on transmission: endemic, highland endemic prone, arid seasonal and low risk. Diversity in risk in the zones is determined largely by altitude, rainfall patterns and temperature. 29% of the population lives in

malaria endemic zones where children and pregnant woman bear the brunt of the disease. The seasonal transmission of malaria in the arid and highland epidemic prone areas confers negligible immunity to malaria, making the whole population vulnerable to malaria (HMIS, 2008).

Malaria transmission depends upon the presence of the *Plasmodium* parasite, suitable vectors and susceptible human hosts. Existing evidence suggests that micronutrient deficiencies and general under-nutrition increase the burden of malaria morbidity and mortality. In Kenya, 76 % of children are vitamin A deficient and there is high risk of zinc deficiency in about half of children and mothers although its magnitude in the Kenyan population is still not clear due to insufficient data available (Mwaniki *et al.*, 1999). Large numbers of children suffer and die of malaria due to nutritional inadequacies in terms of protein energy, zinc and vitamin A (Fishman *et al.*, 2004).

Widespread zinc and vitamin A deficiencies in malaria endemic regions contribute to growth faltering and compromise a child's ability to fight infection (Simmer *et al.*, 1988).

A trial study to investigate the ability of vitamin A and zinc to help boost natural immunity to malaria was carried out in Wosera area, Maprik District in Papua New Guinea (Shankar *et al.*, 2000). Regular vitamin A and zinc supplementation appeared to be complementary in decreasing the burden of malaria in children. Vitamin A reduced by more than a third the febrile illnesses due to mild moderately high levels of malaria

parasites in children and significantly reduced spleen swelling, an indicator of chronic malaria. However, it had little influence on the worst cases, where children had a very high number of parasites in their blood. Zinc, on the other hand, helped blunt the severity of the worst cases. As a result, there were over a third fewer malaria cases seen at health centers among those given a placebo. In addition, overall clinic visits by those children who had received zinc decreased by a third, and signs of other infections (cough and diarrhoea) were reduced by 20-50 %.

1.2 Statement of the Problem

Malaria poses a major problem for developing countries mainly because of breakdown of malaria control programmes, the constant emergence of drug resistant parasites, and possibly climatic changes. Available evidence indicates that malnutrition is associated with increased occurrence of infection and symptomatic malaria, and considerably higher likelihood of malaria mortality in humans. Nutritional interventions using micronutrients may not only increase nutritional status of children but also reduce malaria-associated complications (Shanker *et al.*, 2000).

Although some studies have shown positive results others have failed to detect any association between nutritional status and malaria susceptibility (Nyakeriga *et al.*, 2004; Muller *et al.*, 2003, little has been done on the same in malaria endemic regions like Kisii district.

1.3 Justification for the Study

Across the world, commitment is growing to reduce or even eliminate the Incidence of malaria. The concern is rooted in both humanitarian and economic issues. Malaria is responsible for extensive mortality and morbidity, especially of children, and it saps the vitality of the workforce and diverts resources needed for development. Kenya's response to the impact of the disease has been multifaceted guided by the (HMIS 2008). By demonstrating that there is a relationship between serum zinc and vitamin A status in children and susceptibility to malaria, the findings of this study will be used as a basis for nutritional interventions with a view to reducing morbidity and mortality in children. This study should be able to redirect emphasis from focusing on the vector to considering host factors in malaria control efforts. Empowering the human host to fight the Plasmodium parasite is likened to saving the children upstream before falling into the river of malaria sickness.

1.4 Null Hypothesis

There is no difference in serum levels of retinol and zinc between children with and without malaria parasitaemia in Kisii District, Kenya.

1.5 Objectives

1.5.1 General Objective

To determine serum retinol and zinc levels in school children with and without malaria parasitaemia in Kisii District.

1.5.2 Specific Objectives

- To establish the nutritional status of children with and without malaria parasitaemia.
- To determine the frequency of food intake by children with and without malaria parasitaemia.
- To determine clinical signs and full haemogram profiles of children with and without malaria parasitaemia.
- To determine serum retinol and zinc levels in children with and without malaria parasitaemia

CHAPTER TWO

LITERATURE REVIEW

2.1 Malaria Transmission.

The *Plasmodium* parasite spends part of its life in humans and the other part in mosquitoes. The parasite is transmitted from one person to another through the bite of a female *anopheles* mosquito, which requires blood for its reproduction cycle (Abraham and James, 1995).

The malaria parasite enters the human host when an infected *anopheles* mosquito takes a blood meal. Inside the human host the parasite undergoes a series of changes as part of its life cycle. Its various stages allow plasmodia to evade the immune system, then infect the liver and red blood cells and finally develop into a form that is able to infect a mosquito again when it bites an infected person. Inside the mosquito, the parasite matures until it reaches the sexual stage where it can again infect a human host when the mosquito takes its next blood meal, 10 to 14 or more days later (Gillies, 1972). Details of the life cycle of malaria are shown below in figure 1.

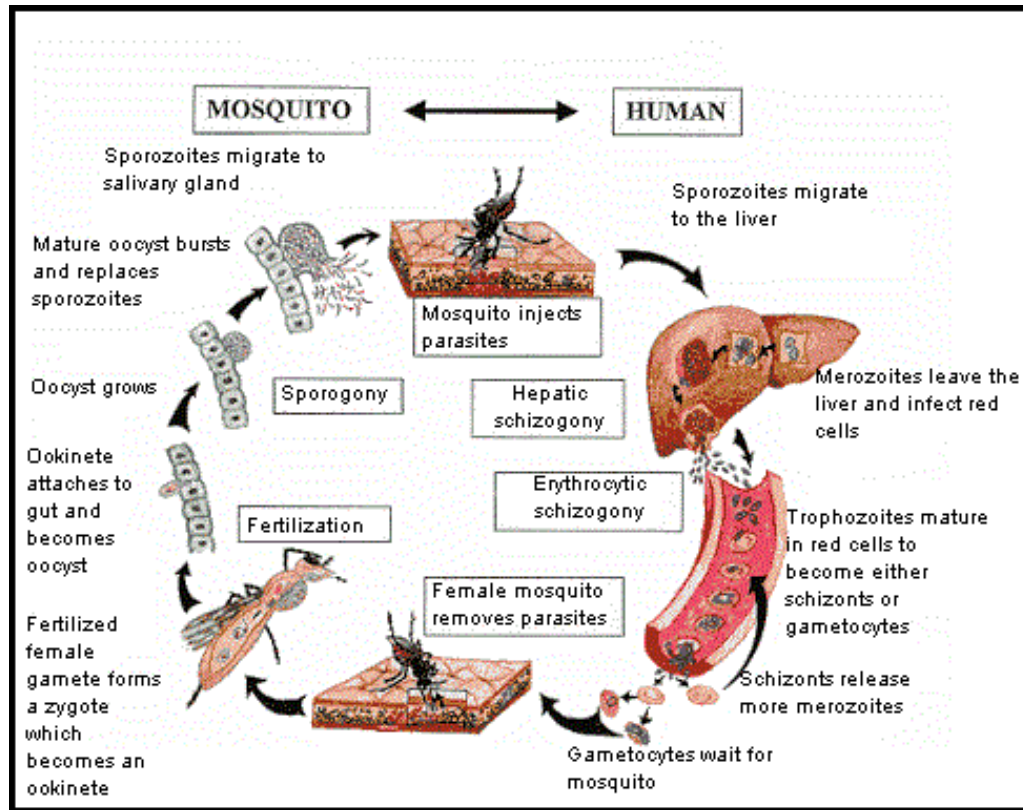


Figure 1: The life cycle of malaria

Malaria symptoms normally appear about 9 to 14 days after the bite of an infected mosquito, although it varies with different *Plasmodium* species. Malaria produces flu-like symptoms including chills, fever and sweating accompanied by nausea, vomiting, headache and other types of symptoms. If not treated or if the parasites are resistant then the infection can progress rapidly to become life threatening. It can kill by infecting and destroying red blood cells (cerebral malaria) or other vital organs (Holding *et al.*, 1999).

2.2 The devastating impact of Malaria

Malaria is a public health problem in many parts of the world. In 2003 approximately 40 % of the world's population and mostly those living in world's poor countries were at risk of malaria. In the tropical and sub-tropical regions malaria causes more than 300-500 million acute illnesses and at least one million deaths annually. Ninety percent of these deaths due to malaria occur in Africa south of Sahara mostly among young children (Snow *et al.*, 1999).

Malaria causes poverty, and is not just a result of it. Regions that have eradicated malaria have substantially higher economic growth rates than neighbouring regions. Malaria affects the demography of a region by increasing infant and child mortality, thus preventing a large proportion of the population from reaching working age. Increased child mortality also leads to increased fertility as women have more children to compensate for those lost to malaria (Murphy and Breman, 2001).

Adults living in malaria endemic regions develop immunity to its symptoms. Pregnancy reduces a woman's immunity. Anemia prevalence in pregnant African women is high. The main factors causing anemia are deficiencies of iron and possibly folate induced by inadequate diet, malarial and hookworm infections. Severe malarial anemia resulting in hemorrhage is a major cause of death among pregnant women (David *et al.*, 2002).

Low maternal hemoglobin strongly predicts pre-term delivery and low birth weight. Infants with low birth weight are significantly more susceptible to other infections and

have a higher risk of dying during infancy. Those who survive are at a greater risk for poor growth and development (McGregor, 1984).

Malaria creates a huge economic burden because of hospital admissions, national malaria control programmes and protection from mosquitoes, the cost to individuals of anti-malarial drugs, treatment, and lost wages. Many malaria sufferers cannot afford or have no access to medical treatment. Children lose time from school and suffer throughout life from effects on cognitive development and education levels attained. Malaria limits foreign investment, tourism, transport systems, internal movement of labour and commerce. Attracting educated people to malaria endemic regions is difficult, limiting the viability of areas with development potential and rich natural resources. Because malaria strikes during the rainy harvest season, when worker productivity needs to be at its highest, the disease can harm food security and agricultural production (Shepard *et al.*, 1991)

Malaria is a major cause of morbidity and mortality in tropical and subtropical regions. It often afflicts populations that are both impoverished and malnourished, and a large proportion of the burden of malaria falls upon the most vulnerable within the population, children and pregnant women (Shankar *et al.*, 2000). A variety of interventions are used to combat malaria, including insecticide-treated bed nets, environmental control, chemoprophylaxis, and prompt, appropriate case management. There exists no single

solution or program to combat malaria, rather a comprehensive approach is required with concurrent interventions on many levels.

Early diagnosis and prompt malaria treatment are the mainstays of the current approach to malaria control. The limited efficacy of anti-malarial medication and the potential of the malaria parasite to develop resistance towards them hinder this approach. Second line drugs are usually required to deal with the resistance at the high cost of alternative treatment; the greater potential for adverse effects and limited availability in some areas restrict their utility for most susceptible populations in endemic areas (Jones *et al.*, 2003).

While nutrition plays a major role in maintaining health, malnutrition appears to generate vulnerability to a wide variety of diseases and general ill health (Semba and Bloom, 2001). Opinions are mixed regarding how under-nutrition, whether it is characterized in terms of growth faltering or micronutrient malnutrition, affects susceptibility to malarial illness and mortality. Historical observational studies provide some evidence of harm resulting from adequate nutrition, whereas more recent studies indicate either no evidence of benefit or some benefits resulting from nutritional adequacy. Animal studies suggest that improved nutritional status is protective against malaria, but consensus has yet to be reached regarding its effects in human populations (Shankar, 2000).

2.3 Zinc

2.3.1 Role of zinc

Zinc is an essential trace element for all forms of life. The benefits of zinc have been appreciated for many years; generations of mothers have applied zinc cream to cure nappy rash and it is common knowledge that zinc helps heal wounds (Berg and Shi, 1996).

Clinical deficiency in humans was first described in 1961, when the consumption of diets with low zinc bioavailability due to high phytic acid content was associated with adolescent nutritional dwarfism in the Middle East. Since then, zinc insufficiency has been recognized by a number of experts as an important public health issue especially in developing countries (Prasad, 1979). amounts of 2 gm or more per day, zinc sulfate (most available form of zinc) can cause toxicity symptoms such as nausea, vomiting, epigastric pain, diarrhea, lethargy and fatigue (Fosmire, 1990). Chronic consumption of high levels of zinc may induce copper deficiency or aggravate marginal copper deficiency as well as make alterations in the immune response and serum lipoprotein levels (Chandra, 1994; Prasad *et al.*, 1978).

Numerous aspects of cellular metabolism are zinc- dependent. These can be divided into three categories namely; catalytic, structural and regulatory roles.

Nearly 100 different enzymes depend on zinc for their ability to catalyze vital chemical reactions. Zinc-dependent enzymes can be found in all known classes of enzymes (Vallee and Falchuk, 1993).

Zinc plays an important role in the structure of proteins and cell membranes. A fingerlike structure, known as a zinc finger motif, stabilizes the structure of a number of proteins. Loss of zinc from biological membranes increases their susceptibility to oxidative damage and impairs their function. Zinc acts as an antioxidant that stabilizes membranes (Fraker *et al.*, 2000).

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). Zinc is important in cell signaling and has been found to influence hormone release and nerve impulse transmission (Vallee and Falchuk, 1993).

Recently zinc has been found to play a role in immune response, neurological function, reproduction, apoptosis (gene-directed cell death) - a critical cellular regulatory process with implications for growth and development, as well as in a number of chronic diseases. Zinc is essential for the synthesis of retinol-binding protein, which is required for mobilization of vitamin A from the liver (Prasad *et al.*, 1978).

2.3.2 Bioavailability of zinc

Zinc bioavailability is relatively high in red meat, eggs and seafood because of the relative absence of compounds that inhibit zinc absorption and the presence of certain amino acids (cysteine and methionine) that improve zinc absorption. The zinc in whole grain products and plant proteins is less bioavailable due to their relatively high content of phytic acid, a compound that inhibits zinc absorption (King *et al.*, 1999). Yeast

reduces the level of phytic acid in foods making leavened whole grain breads to have more bioavailable zinc than unleavened whole grain breads. Nuts and legumes are relatively good plant sources of zinc.

2.3.3 Distribution of zinc in the body

Zinc is present in all organs, tissues, fluids, and secretions in the body. However most zinc is located in the fat mass, with about 30 mg zinc/kg tissue, almost all of which (>95 %) is intracellular. Due to the bulk of skeletal muscle and bone in the body, zinc in these tissues accounts for the majority (83 %) of whole body zinc (Iyengar, 1998). When total body zinc content is reduced during depletion, the loss of zinc is not uniform across all tissues. Skeletal muscle, skin, and heart zinc are maintained, while zinc levels decline in bone, liver, testes and plasma (Jackson *et al.*, 1982).

2.3.4 Requirements of zinc in children

In healthy adults, metabolic studies indicate positive zinc balance with intakes of 12.5 mg/day from mixed diet. Therefore, the Recommended Daily Allowances (RDA) was set at 15 mg/day for adolescents and adults, plus 5 mg and 10 mg additional during pregnancy and lactation respectively. For preadolescents the requirement is estimated at 6 mg/day but there are greater dermal losses and more variation (Food and Nutrition Board, 1989).

2.3.5 Zinc deficiency

Zinc deficiency most often occurs when zinc intake is inadequate or poorly absorbed, when there are increased losses of zinc from the body, or when the body's requirement for zinc increases. Signs of zinc deficiency include growth retardation, hair loss, diarrhoea, delayed sexual maturation and impotence, eye and skin lesions, and loss of appetite (Ronaghy *et al.*, 1969). There is also evidence that weight loss, delayed healing of wounds, taste abnormalities, and mental lethargy can occur.

Groups at increased risk of zinc deficiency include those with high requirements for whom other factors make it difficult to acquire diets with adequate zinc content. These include pre-term infants, small-for-gestational-age term infants, young children after the period of exclusive breastfeeding, children presenting with and recovering from malnutrition, adolescents, pregnant and lactating women, and the elderly (King, 1996; Brown *et al.*, 2001).

2.3.6 Zinc toxicity

Individuals may be exposed to high intakes of zinc either through supplements or by contact with environmental zinc. Taken in amounts of 2 gm or more per day, zinc sulfate (most available form of zinc) can cause toxicity symptoms such as nausea, vomiting, epigastric pain, diarrhea, lethargy and fatigue (Fosmire, 1990). Chronic consumption of high levels of zinc may induce copper deficiency or aggravate marginal copper

deficiency as well as make alterations in the immune response and serum lipoprotein levels (Chandra, 1984; Prasad *et al.*, 1978).

2.3.7 Zinc and immunity

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 (Shankar and Prasad, 1998).

Zinc plays an important role in specific immune defenses such as humoral and cell-mediated immunity. The humoral immune response relies on production of immunoglobulins by B-lymphocytes. The cell mediated immunity relies on T-lymphocytes, which mature in the thymus gland. B- and T- lymphocytes together with specialized proteins destroy specific microbes. Even a mild reduction of circulating zinc is associated with reduced T cell production of certain critical proteins called cytokines which regulate immune response and act as growth factors for the immune system (Duchateau *et al.*, 1981). Zinc also has a role in normal cellular replication and growth which can affect the rapidly proliferating cells of the immune system.

Supplementation with zinc showed no effect on various malarial indices in a community based cross-sectional surveillance, it was found to reduce visits to health facilities for *P. falciparum* febrile episodes defined as fever with parasitaemia in Papua New Guinea and the Gambia (Shankar *et al.*, 2000). Another trial in Burkina Faso found no effect of zinc supplementation on rates of fever in community-based surveillance of malaria (Muller *et al.*, 2001).

The therapeutic effect of zinc given as an adjuvant to standard therapy during acute malaria in a large double blind, randomized placebo-controlled clinical trial across 5 countries in Africa was evaluated. Despite an improvement in mean serum zinc levels there was no effect on the median time to reduction of fever, parasitaemia or a change in hemoglobin concentration in the initial 72 hours or during the four week follow up (Caulfield and Black, 2004)

2.4 Vitamin A

2.4.1 Nature and sources of Vitamin A

Vitamin A is a generic term for compounds having similar biologic activity. These compounds include retinol, retinal and retinoic acid. The term retinoids refers to both the natural forms of retinol and its synthetic copies. Chemically retinol is a primary alcohol of high molecular weight. Retinal is the aldehyde form of retinol derived by the enzymatic splitting of absorbed carotene. Vitamin A is fat-soluble but insoluble in water and stable during general cooking (Williams, 1994).

In western countries, the predominant source of vitamin A in the diet is pre-formed vitamin A (*all-trans* retinol), which is derived from animal products such as milk, butter, cheese, egg yolk, liver and some fatty fish. In addition to the pre-formed vitamin, vitamin A can also be derived from provitamin A compounds produced by plants. The main sources of provitamin A are dark- green leafy vegetables and yellow and orange coloured fruits and vegetables. In most tropical countries, where consumption of animal

products is relatively low, the main sources of vitamin A activity in the diet are the carotenes particularly β -carotene which has a structure identical with retinol in both halves of the molecule (Booth *et al.*, 1992). A few other carotenes also have vitamin A activity. For example, α -carotene which is present together with β -carotene in red palm oil has a structure identical to retinol in only one half of the molecule and has half of the vitamin A activity of β -carotene.

2.4.2 Vitamin A bioavailability

It is thought that the conversion of provitamin A to retinol occurs in the gut wall and that retinol in the small intestine is taken up by absorptive cells of the small intestine. The amount of (pro) vitamin A absorbed from the gut and which becomes available to tissues is referred to as bioavailability. The absorption of retinol is probably more than 80 % while that of carotenes is lower and is affected by various factors which include species of carotenoid, molecular linkage, amount of carotene consumed in a meal, matrix in which the carotenoid is incorporated, absorption modifiers, nutrient status of host, genetic factors, and interactions between the other factors (Booth *et al.*, 1992).

2.4.3 Absorption, transport and storage of vitamin A.

Route of absorption of vitamin A and carotene parallels that of fat. In the intestinal mucosa, all the retinol from both preformed animal sources and from plant carotene conversion is re-esterified with long chain fatty acids. Incorporation into chylomicrons enables it to be transported into the bloodstream via the lymphatics and is carried to the

liver for storage and distribution as needed by the body cells. In the liver, the retinol is transported when bound to retinol-binding-protein (RBP) which complexes with serum prealbumin. RBP transports vitamin A in the circulation and then may be removed from circulation by the kidney (Rodriguez and Irwin, 1980). Most body vitamin A (up to 90 %) is stored in the liver as esters of long-chain fatty acids but also in small amounts in the kidneys, lungs and fat tissue. Liver stores and plasma levels of vitamin A are reduced during periods of infectious disease. Vitamin A metabolism is disturbed in hepatic cirrhosis and malabsorption. Chronic use of mineral oil as a laxative hinders vitamin A absorption.

2.4.4 Recommended Intakes

The current recommended nutrient intakes (RNI) of vitamin A published by the FAO/WHO are two-tiered (FAO, 1988), with a basal level corresponding to a recommended intake to prevent deficiency, and a safe level similar to the recommended dietary allowance (RDA) set for the United States (NRC, 1989) which corresponds to an intake that provides for adequate liver storage of this fat-soluble vitamin (Olson, 1987). The basal level for adult ranges from 270 to 400 retinol equivalents (RE), whereas the recommended safe level corresponds to 500 to 600 RE, with additional recommended intakes of 100 RE during pregnancy and 350 RE during lactation (FAO, 1988).

2.4.5 Vitamin A deficiency

Vitamin A deficiency may occur due to inadequate dietary intake, poor absorption due to lack of bile or defective absorbing surface, and inadequate conversion of provitamin A carotenoids because of liver or intestinal disease (Sommer, 1995). Vitamin A deficiency is rare and most vitamin A associated morbidity results from mild to moderate deficiency. Conditioned deficiencies occur when a bodily dysfunction interferes with absorption, storage, or transport of vitamin A as in malabsorption due to bile acid insufficiency, protein-energy malnutrition, liver disease or abetalipoproteinemia.

Studies in animals indicate that the rate of vitamin A metabolism is determined by supply. As a deficit develops, the animal slows the rate of vitamin A metabolism in an attempt to conserve the available supply. Prolonged deficiency may produce skin changes, night blindness and corneal ulcerations. In extreme states, the mucous membranes of the respiratory, gastrointestinal, and genitourinary tracts may be affected. Vitamin A deficiency alters amino acid patterns in tissues and plasma and enhances the excretion of urea and taurine (Bates, 1995).

2.4.6 Toxicity of vitamin A

It is possible to consume a potentially toxic quantity as many persons take additional megadose supplements and the liver can store large amounts of vitamin A.

Hypervitaminosis A has been observed in adults and children taking in excess of 50000 I.U. and 25000 I.U. daily respectively for several years (Wason, 1982). This is manifested by joint pain, thickening of long bones, loss of hair and jaundice. Excess vitamin A may also cause liver injury with resulting portal hypertension and ascites.

2.4.7 Functions of vitamin A

Once retinol is transferred to the target tissues, it is involved in several biochemical/physiological processes. A number of the functions of vitamin A can be met only by retinol while others can utilize other retinoids. Those functions which only retinol can perform include vision at low light intensities, synthesis of “active sulphate” which is an important intermediate in synthesis of mucopolysaccharides, and in reproduction where the effect is probably mediated through the synthesis of steroid hormones (Brown *et al.*, 2001).

Those reactions in which retinoids participate include: involvement in cellular differentiation, synthesis of glycoproteins (the effect of retinoids being mediated through their involvement in the glycosyl transfer reaction), the synthesis of RNA and hence also the synthesis of DNA and proteins, immunity, growth and inhibition of cancer (Bates CJ, 1995). In addition, it is thought that vitamin A is also involved in haemotopoiesis, gene expression and perhaps nitrogen metabolism in cells.

The retinoids (except for retinoic acid) are necessary to support normal sexual maturation during adolescence and function of adult reproductive system. Vitamin A deficiency causes glandular degeneration and sterility (Williams, 1994).

Vitamin A plays an essential role in the immune response and in eye health. The eye's ability to adapt to changes in light depends on a light-sensitive pigment – rhodopsin (commonly known as visual purple) in the rods of the retina. Rhodopsin is composed of retinal and the protein scotopsin. In the cones, retinal combines with photopsin to form the three pigments responsible for colour vision. When the body is deficient of vitamin A, the normal rhodopsin cannot be made and the rods and cones of the retina become increasingly sensitive to light changes, causing night blindness (Williams, 1994). The dominant symptom of severe vitamin A deficiency is xerophthalmia, a major cause of blindness in Africa and Latin America that initially appears as night blindness and results in corneal ulceration and blindness if left untreated. Vitamin A supplementation has been shown to improve general eye health, as well as decrease measles, diarrhea, and all-cause mortality (Semba, 1998).

Vitamin A plays an essential role in the proper functioning of the immune system and is believed to be necessary for host resistance to malaria, although early animal studies suggested that deficiency was protective (Shankar, 2000). In 1946, a study of vitamin A-deficient chicks indicated severe vitamin A deficiency was associated with slightly milder infection with malaria compared with well-nourished chicks, while the same

experiment with ducks was unable to demonstrate an association (Rice *et al.*, 2004). Later studies in rats indicated that vitamin A deficient rats were significantly more susceptible to the rat malaria parasite *P. berghei* than were those rats with adequate vitamin A intake (Ross *et al.*, 1946). Follow up studies, however, were less convincing and only able to demonstrate increased susceptibility to malaria in those rats with very severe vitamin A deficiencies that began when the rats were very young (Krishnan *et al.*, 1976). Overall, animal studies suggest that vitamin A deficient animals are more vulnerable to malaria morbidity and mortality.

The hypothesized mechanism through which vitamin A mediates susceptibility to malaria is increased phagocytosis of parasitized erythrocytes and reduced proinflammatory cytokine responses to infection. Vitamin A may assist in the up-regulation of CD36 expression, which aids in phagocytosis and may activate substances (peroxisome proliferators-activated receptor), which inhibit the inflammatory responses associated with severe and cerebral malaria (Stoltzfus *et al.*, 1989).

Cross-sectional studies suggest an inverse relationship between plasma retinol concentrations and increased malaria parasitemia, but the causality of the association is uncertain (Friis *et al.*, 1997).

A clinical trial in Papua New Guinea found a significantly lower risk of malaria morbidity in the vitamin A supplemented group compared with the placebo group. A clinical trial in Ghana found no association between vitamin A supplementation and

malaria morbidity or mortality. However, this study did not have the statistical power to detect a difference of less than 70 % between the two groups (Rice *et al.*, 2004).

Studies have shown that the fraction of malaria morbidity attributable to vitamin A deficiency was 20 % worldwide. More than 90 % of the 187,000 malaria deaths worldwide attributable to vitamin A deficiency occur in Africa (Serghides and Kain, 2002).

A prospective longitudinal study carried out among 3,400 children in West Java Indonesia, showed that children with mild vitamin A deficiency developed respiratory disease twice and diarrhoea three times as frequently as non-xerophthalmic controls (Sommer *et al.*, 1984). Studies by other groups have also shown a relationship between mild signs of vitamin A deficiency and childhood morbidity in the developing world setting. Vitamin A supplementation reduced mortality from measles and other infectious diseases (Tidsskr, 2000). The effect of Vitamin A on infection may be mediated through two major ways namely, improving the epithelial repair and through immunological protection.

2.5 Malaria and Iron status

Anaemia is the most common consequence of malaria infection in both immune and semi-immune individuals. The impact of malaria infection on anaemia is most marked in young children and pregnant women, especially in primigravidae. This is probably related to a lower level of malaria-specific immunity in these groups. A study on

malaria chemoprophylaxis given to pregnant women in the Gambia showed a significant increase in packed cell volume (PCV) only in primigravidae, although parasite rates were also significantly reduced in multigravidae (Alonso *et al.*, 1994). Similarly, a placebo-control trial on vaccinating Tanzanian children between 1 and 5 years old with the SPf66 antimalarial vaccine showed no difference between the placebo and control groups with respect to PCV levels, despite a significant reduction in both the incidence of clinical malaria and parasite density (Alonso *et al.*, 1991). However, preliminary results of a descriptive study of malaria risk among infants from the same area suggest a negative correlation between clinical malaria episodes and PCV level (Menéndez *et al.*, 1994). This would imply that the impact of malaria on anemia correlates inversely with the level of immunity against the infection.

Defective red cell production has been observed mainly in children with severe anaemia, low reticulocyte count and low parasitaemia (Abdalla, 1990).

Malaria parasites may affect iron status through the following mechanisms: reducing intestinal iron absorption, sequestering iron within the malarial pigment hemozoin, consuming iron for its own metabolism, promoting/stimulating the mobilization of iron to body stores and releasing iron into the circulation during intravascular haemolysis (Greenwood *et al.*, 1989).

Iron, vitamin A and zinc have a similar distribution in the food supply, and some of the same food components similarly affect the absorption of these nutrients. High rates of iron-deficiency anemia may be used as suggestive evidence of the risk of zinc

deficiency. Adequate dietary protein and zinc appear to be necessary for retinol mobilization (Solomons and Russel, 1980).

A positive correlation between anemia and indicators of the risk of zinc deficiency has been demonstrated (Folin *et al.*, 1994). A lack of relationship between serum zinc and haemoglobin has been reported in some populations, such as young Vietnamese children (Thu *et al.*, 1999). This may in part be attributed to the effect of confounding factors such as the presence of concurrent infections, including malaria, on the biochemical indices. Thus, the occurrence of anemia does not necessarily indicate the presence of zinc deficiency.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Design

This was a case-control study. Of the 111 school children recruited for the study, 55 had malaria parasitaemia (cases) while 56 of them did not have (controls). Serum zinc and vitamin A levels of the two groups were established and compared.

3.2 Study Site

The study was conducted in Kisii District, Nyanza Province, Western Kenya. In 2009, Kenya's projected population was approximately 39.4 million, a little over 10 million more than the 1999 population (KNBS, 2008). Children under five years of age comprise 17 per cent of the total population, while a quarter of the entire population consists of women of reproductive age (15 to 49 years). Kenya's infant mortality rate (IMR) is 52 per 1000 live births, under 5 years mortality is 74 per 1000 live births and the maternal mortality ratio (MMR) is 410 per 100,000 live births. 22.9 % of the under 5-year-olds are malnourished (KDHS, 2008).

Kisii District is the most densely populated area of Nyanza Province with 1691707 people and ranks second in terms of rural population density in Kenya after Vihiga. Kisii has the least unemployment in Nyanza at 5 % and it also has the second highest mean household income in the province after Kisumu. Over half of Kisii residents have clean

drinking water and safe sanitation. Water is easily available from rivers, wells, springs, roof catchments and boreholes, although the only treated water supply is found in Kisii Municipality (KNBS, 2009).

Kisii District lies on a highland equatorial climate, and as such it receives rain almost throughout the year, although there are two rainy seasons (March to May and October to November). Average rainfall is over 1500 mm and is quite reliable, helping to support cash crops (coffee, tea, and pyrethrum) and subsistence crops (maize, beans, millet, potatoes).

The major diseases in Kisii are malaria, anaemia, pneumonia, meningitis, tuberculosis, measles and gastroenteritis. Infant mortality has been declining for several years and the acceptance of immunization programs is increasing, but a large proportion of children are believed to be stunted due to poor nutrition (KDHS, 2008).

This is an epidemic malaria eco-zone area with extremely high transmission and prevalence of over 70 % among children aged five years and below (Murphy and Breman, 2001). This area experiences malaria epidemics, which have almost become an annual event during the last decade. Epidemics are experienced when climatic conditions favours sustainability of minimum temperatures around 18°C. This increase in minimum temperatures during the long rains favours and sustains vector breeding, resulting in increased intensity of malaria transmission. The whole population is

vulnerable and case fatality rates during an epidemic can be up to ten times greater than those experienced in regions where malaria occurs regularly (NMS,2009). This pattern has been attributed to among others climatic changes, vectoral consequences, changes in land use, decreasing resources for malarial control and treatment, and growth in population movement (Lindsay and Martens, 1998). Recent evidence shows an increase in the number of epidemics in highland areas as well as a spread of epidemic malaria into deeper highland fringes (Githeko *et al.*, 2000).

Health facilities are inadequate, unevenly distributed and lacking in essential medicines. There are two hospitals; the government-run facility in Kisii Town and the Mission Hospital in Tabaka with a bed occupancy rate of 160.3 % (MOH, 1999).

3.3 Study Population

3.3.1 Inclusion Criteria:

- Child aged 6-10 years.
- Child did not suffer from any chronic illness.
- Child must not have taken zinc and vitamin A supplements in the past one month.

3.3.2 Exclusion Criteria:

- Child aged below 6 years because they experience erratic growth and also numerous nutritional studies have been carried out in this age bracket.

- Child older than 10 years as puberty begins and hormonal influences accompanied with enormous physical changes might interfere with findings.
- Child with chronic illness(es) and recent supplementation might interfere with micronutrient levels.

3.4 Determination of Sample Size

Drawing from experiences of a study conducted in Kenya (Mbakaya *et al.*, 2004), the following standard formula from the Sample Size Determination Manual (Lemeshow and Lwanga, 1991) was used to calculate the sample size for the study.

$$\begin{aligned}
 n &= \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2 2\delta^2}{d^2} &= & \frac{21 \delta^2}{d^2} \\
 & &= & \frac{21 (14)^2}{10^2} \\
 & &= & \mathbf{41.16} \\
 & & & \text{====}
 \end{aligned}$$

where,

$$Z_{1-\alpha/2} = 1.96$$

$$Z_{1-\beta} = 1.28 \text{ for } \beta = 10\% \text{ (i.e. 90 \% power of study)}$$

$$\text{Variance} = \delta^2 = \text{sd}^2 = 14^2 \quad \text{and,}$$

$$d = \text{difference to be detected} = \mu_1 - \mu_2 = 10 \text{ } \mu\text{g/dl serum zinc}$$

- Note: Using serum retinol with a mean of 0.75 $\mu\text{g/dl}$ and standard deviation of 0.28 $\mu\text{g/dl}$, and difference detected (d) of 0.25, a sample size of 26 was yielded.

This is less compared to using serum zinc mean levels whereby a sample size of 42 cases and a similar number of controls were found to be adequate.

3.5 Sampling Procedure

With the help of the vector control unit in Kisii District Hospital and guided by accessibility of the schools, seven primary schools were purposively selected from areas prone to malaria infection in the larger Kisii District (Table 1).

The local administration and head teachers of the selected schools helped to inform guardian/parents (primary caretakers) about the study. They were requested to allow their children to participate after they were informed about the study procedures, especially the collection of blood samples. Children with written informed consent (Appendix 3) from parents/guardians were enrolled for the study.

A total of 1200 pupils who passed the inclusion criteria and with written consent of guardians/parents, were tested for malaria parasites.

For each child testing positive with malaria parasitaemia (case), the next one testing negative with malaria parasitaemia (control) was recruited. After exhausting the number of eligible children in a school, the exercise continued to the next one till the expected calculated sample size was obtained.

A total of 111 pupils (55 with malaria and 56 without malaria) were recruited from 7 primary schools for the study. All recruits were residents of 3 newly sub-divided districts of the larger Kisii district namely; Gucha, South Kisii and Nyamira (Table 1).

Table 1: Distribution of study population by school and district.

School	District	N
Mosache primary	Gucha	12
Mwata primary	South Kisii	40
Genga primary	South Kisii	12
Nyandiwa primary	Gucha	8
Nduru D.E.B	Gucha	4
Metembe primary	Nyamira	3
Mugori primary	South Kisii	32
Total		111

3.6 Data Collection

3.6.1 Study Questionnaire

Parents/guardians of the enrolled children completed a structured questionnaire, which included demographic data, information on the use of the anti-vector measures to prevent malaria, recent anti-malarial use, and significant medical history (Appendix 4).

A clinical estimate of age to the nearest year was used in this study as some parents/guardians could not exactly remember when their children were born and some of the dates and months of birth were not documented in class registers.

3.6.2 Food Intake Frequency

The dietary history method, recalling past intake of individuals, is a technique of estimating usual dietary intake (Burke, 1947; Young, 1965). Using information on food dietary habits of the population under study, a locally adapted food frequency questionnaire (FFQ) was drawn (Appendix 5). The FFQ listed only foods most commonly consumed in the area, rather than all possible foods. Food was categorized into 5 broad groups namely; cereal grains and tubers and plantains, animal and dairy products, legumes, vegetables, and fruits. Guardians/parents were asked the number of times they fed their children on the various foods in the past 7 days. Frequency distribution of food items (dietary diversity) consumed from the 5 broad food groups in the past 7 days, was done.

3.6.3 Clinical signs

3.6.3.1 Heart/pulse rate

A trained nurse took the pulse rate by palpation of the carotid artery for one full minute in order to evaluate rhythm and volume accurately (Roper *et al.*, 1990). Since a diurnal rhythm is noted in pulse rate, for uniformity, this procedure was done between 10.am to 1pm in all schools.

3.6.3.2 Respiratory rate

The pupils were asked to sit upright and instructed to breathe slowly and deeply through the mouth. Auscultation of the chest was done and a stethoscope was used to determine the frequency, intensity, duration, and quality of the sounds.

3.6.3.3 Blood pressure

After settling down the children, blood pressure (Bp) was measured by auscultation using a mercury sphygmomanometer, a stethoscope, and a blood pressure cuff. Malasarioris *et al.*, 1981, categorized normal Bp values for children aged 6,8,and 10 years as 100/60 mm Hg, 105/60 mmHg and 110/60 mmHg respectively. For the purpose of this study, children with the diastolic value >60 mmHg were considered to have higher than normal blood pressures.

3.6.3.4 Temperature

A trained nurse recorded the auxiliary temperature over a period of 3 minutes using a clean glass thermometer that had been shaken down below 360 °C. A temperature of >37.5 °C or lower than 36 °C was considered abnormal, suggesting a problem had arisen with the body temperature controlling mechanism (Roper *et al.*, 1990).

3.6.4 Nutrition status

3.6.4.1 Anthropometric measurements

Weight, height, and left mid upper arm circumference were measured by trained health workers in duplicate following standard procedures (WHO, 1983) to assess the nutritional status of the children.

Weight to the nearest 100 g was measured with the children barefoot and wearing light clothing using an electronic load cell model (Seca 779, Germany).

Standing height was measured to the nearest 0.1 cm without footwear and after removal of headgear (Hautvast *et al.*, 2000).

Physical measurements reflect the total nutritional status over a lifetime. Height reflects past nutrition or chronic nutritional status. MUAC and weight reflect present nutritional status and are used to assess the skeletal energy reserves both as fat and as protein (Krause and Mahan, 1984).

Because children are growing, their overall nutritional status is calculated in terms of standard deviation (SD) scores or Z-scores in which the placement of a measure (a child's weight or height) within a distribution (the reference weights of healthy children) is characterized by the distance from the median in SD units (Lohman *et al.*, 1988). Z-scores at the lower end of the distribution are categorized in nutritional terms as mild (-1.01 to -2.00 SD), moderate (-2.01 to 3.0 SD), or severe (<-3.0 SD).

WAZ, HAZ, and WHZ scores were calculated using the WHO/NCHCS cut off points (WHO, BASIC, UNICEF, 1999). Children were classified as stunted, underweight, or wasted if the HAZ, WAZ, or WHZ was <-2, respectively.

3.6.4.2 Body Mass Index (BMI)

The weight and height measures were used to calculate the child's body mass index: weight (kilograms) divided by height (meters) ². This index has been found to have the least correlation with body height and high correlation with body fatness (Truswell, 1992). BMI was classified as mild between -2 and -1Z scores, moderate between -3 and -2Z scores and severe acute <-3Z scores of the reference population.

3.6.4.3 Mid Upper Arm Circumference (MUAC)

MUAC was measured to 0.1 cm using non-stretchable measuring tapes (Zerfuss International tapes, Ross Ltd, USA). The midpoint of the left upper arm was found by halving the distance between the shoulder and tip of the elbow. The tape was held snugly around the arm at this point with the arm hanging straight.

Each child could be classified as having normal, low, or very low MUAC-for-age based on published age and sex specific cut off values for MUAC representing the -2Z and -3Z-score values of reference population (Cole *et al.*, 1989).

3.6.5 Blood collection and analysis

3.6.5.1 Diagnosis of malaria

Malaria was diagnosed by examining thick blood smears under the microscope for asexual forms of *Plasmodium* noted.

3.6.5.2 Blood collection

A 4-ml blood sample was taken from the children in the non-fasting state between 10 am -12 Noon. Venous blood (2 ml in a trace-element-free tube and 2 ml in an EDTA tube) was taken after cleaning the area of venipuncture with 70 % alcohol and allowing it to dry before inverting the sterile needle into the vein. The blood was immediately transported in an ice box to Kisii District Hospital haematology laboratory, where 2 ml in EDTA tube was subjected to full haemogram procedures and the rest was centrifuged at 2000- Xg for 15 minutes. The resultant serum was covered in foil, frozen at – 20 °C, and transported to KEMRI laboratories for analysis of serum retinol and zinc.

3.6.5.3 Full Haemogram

The values of haemoglobin concentration were interpreted in relation to age-specific and sex-specific reference standards (WHO, 1968). For the purpose of this study, mild anaemia was diagnosed when haemoglobin concentration was between 10 g/dl and 7 g/dl and severe anaemia when it was below 7 g/dl (FAO / WHO, 1985).

3.6.5.4 Vitamin A analysis

Serum retinol concentration were measured by High performance liquid chromatography HPLC (Hitachi, Ltd, Tokyo), a method initially described by Bieri *et al.*, 1979. HPLC has an advantage as interference from retinyl esters can be avoided. Before starting the extraction procedure, 5 µg/L retinyl acetate (100 mg/L) was added to 100 µL serum as an internal standard. The extract was reconstituted with 100 µL mobile phase

(volumetric methanol: water, 95:5) and 20 μL was injected into a guard-fitted, normal-phase stainless steel column (microbondapak C 18,3.9 X 300, mm particle size 10 μm ; Waters Associates, Milford, MA).

Vitamin A status, on the basis of the serum retinol concentration, was classified according to UNICEF- WHO, 1994 criteria as follows; $<1.07 \mu\text{mol/L}$ – vitamin A deficiency, 0.7 to $1.07 \mu\text{mol/L}$ - low marginal vitamin A status, $>1.07 \mu\text{mol/L}$ – adequate.

3.6.5.5 Zinc analysis

Determination of serum zinc was done by colorimetric method using Flame Atomic Absorption Spectrometry (Smith *et al.*, 1979). In this study, a ratio of one unit of serum to 9 units of double distilled water was vortex mixed. This approach is believed to free from interference effects of protein. All measurements were carried at 231.09 nm, using a gas flow rate of 2.4 L/min of analytical grade acetylene and 8 L /min of filtered air and in a background correction mode.

In view of established difficulties in determining precise nutrition status at community level, cut-off for high risk of zinc deficiency was adopted in this study. Since all samples were non-fasting, a cut-off of 65 $\mu\text{g/dL}$ was used in analysis and interpretation (Prasad, 1985; Hotz *et al.*, 2003).

3.7 Data management and analysis

Following well-refined guidelines, clearly structured questionnaires were used to gather demographic data and relevant medical history of the children.

Each questionnaire was carefully checked for inconsistencies after completion.

A data entry screen form resembling the questionnaire was set up and valid values defined before entering data. Data was examined for normality and transformed as appropriate.

Data was entered into a database using Epi info to compute anthropometric indices and analyzed using SPSS version 11.5.

Descriptive statistics such as mean, standard deviation, median and range were used to indicate the average value and spread of continuous variables like education levels and occupation of care givers, frequency of feeding per week, anthropometrical measurements, haematology profile, retinol and zinc levels, and clinical signs.

Independent t-test was used to test for mean comparison of the continuous variables between malaria positive and malaria negative children.

To measure relationships between two continuous variables e.g. retinol and zinc, Pearson's correlation coefficient was applied.

Frequency distribution of categorical variables (socio-demographic indices, categorized serum zinc and retinol levels, clinical signs and anthropometrical measurements) was done.

Cross tabulations between categorical variables and malaria status was also performed to uncover the distribution patterns.

Pearson chi-square test was used to test for association between malaria status and individual categorical variables.

Pie charts were used to show the distribution of individual variables while bar graphs were used to show the association between two categorical variables.

Data was presented using tables and graphs. Scatter plots were used to show the extent of the relationships while box plots were used to show the extent of change between malaria positive and malaria negative children.

3.8 Ethical Considerations

Ethical approval for the study was granted by the KEMRI Ethical Review committee (Appendix 1).

Children were enrolled in the study only after their guardians/parents (primary caretakers) had fully understood facts in the Information Sheet (Appendix 2) and gave their informed consent (Appendix 3). There was no risk to the participants in this study and neither were there any direct benefits. However, the children with malaria parasitaemia were treated using Artemisinin-based Combination Therapy (ACTs) as recommended by the Ministry of Health.

The names of the children were kept private to the extent allowed by law. Access to the study data was limited to the researcher. No personal identities were entered into the computer database.

CHAPTER FOUR

RESULTS

4.1 Socio-demographic characteristics

4.1.1 Gender and age of study population

Gender and age distribution of the children were comparable. The ratio of Male to Female was 1:1, a distribution pattern of 47 % to 53 % respectively. Mean age of study population was 7.7 years. The distribution ratio by gender among malaria positive to negative was 1:1.

4.1.2 Guardian/parent occupation

Majority (89 %) of the guardians were farmers. The rest were either casual workers or business persons (Table 2).

Table 2: Guardian/parent occupation

Guardian occupation	N	%
Farmer	99	89.2
Casual employment	9	8.1
Business person	3	2.7
Total	111	100

4.1.3 Guardian/parent level of education

About 20 % of the parents/guardians had no formal education while majority had attained primary education. Only 9 % had acquired secondary/college education (Table3).

Table 3: Highest level of parent/guardian education

Guardian level of education	N	%
No formal education	22	19.8
Primary	79	71.2
Secondary	6	5.4
College	4	3.6
Total	111	100

4.1.4 Reported morbidity among the children in the past one month.

Reportedly, 49.5 % of morbidity among the children in the past one month was malaria. While 7.2 % of the children reported a combination of malaria and any one form of illnesses (1 scabies, 3 flu/homa, 3 cough, 4 stomach-ache, 1 swollen leg, 1 headache, and 1 fever), 45 % reported no form of illness.

There was no significant association between malaria status and guardian/parent occupation ($P=0.203$), absence from school in the past one month due to illness ($P=0.350$), and child illness status on the day of interview ($P=0.119$). However, there was a significant association between malaria status and guardian/ parent level of

education (0.018). Seventy-three percent of guardians/ parents whose children are malaria positive had no formal education (Table 4).

Table 4: Demographic and health related factors by malaria status of the children.

Variable/ Categories	Malaria status				Odds ratio	P value
	Positive		Negative			
	N	%	n	%		
Guardian's/ Parent's occupation						
Farmer	51	51.5	48	48.5		0.203
Casual employment	2	22.2	7	77.8		
Business man	2	66.7	1	33.3		
Guardian's/ Parent's level of education						
None	16	72.7	6	27.3	3.4	0.018
Primary and above	39	43.8	50	56.2		
Absence from school in the past one month due to illness						
Absent	13	59.1	9	40.9	1.6	0.350
Not absent	42	47.2	47	52.8		
Child's reported illness status on the day of interview (including malaria)						
Sick	12	66.7	6	33.3	2.7	0.119
Not sick	39	43.8	50	56.2		

4.1.5 Use of mosquito control methods by study population.

Slightly more than half of the guardians/ parents used any one of the available mosquito control methods. Among them, 94.9 % used nets, 3.4 % used mosquito coils and 1.7 % used sprays (Table 5).

Table 5: Distribution of malaria by use of mosquito control among study population.

Malaria status	Used mosquito control		Did not use any mosquito control		Total	
	N	%	N	%	N	%
Positive	30	50.8	25	48.1	55	49.5
Negative	29	49.2	27	51.9	56	50.5
Total	59	100	52	100	111	100

4.2 Food Intake Frequency

The number of times food items from the broad 5 groups were consumed by the children in the past 7 days was correlated with malaria parasitaemia (Table 6).

Table 6: Frequency of feeding per week by malaria status

Food groups	Frequency of feeding per week by malaria status						P value
	Malaria Positive			Malaria Negative			
	N	Mean	SD	N	Mean	SD	
Cereals/Tubers/Plantains	55	20	4	56	23	4	0.001
Vegetables	55	17	5	56	17	5	0.936
Fruits	55	9	4	56	10	4	0.169
Animal products	55	10	4	56	12	5	0.037
Legumes	55	4	2	56	5	2	0.028

Higher frequency of feeding on cereals/tubers/plantains, animal products, and legumes was significantly associated with being malaria negative. Mean frequency of feeding on vegetables and fruits in malaria negative and malaria positive children was not significant.

Frequency of dietary diversity ranged from 4 – 5 where majority of the children (95.5 %) consumed 5 types of food per week.

4.3 Clinical Sign

4.3.1 Temperature

No difference was noted in mean temperature between males and females, $P=0.988$. Pooling gender and comparing mean temperature by malaria status, there was no difference between children with and without malaria ($P=0.323$) as shown in Table 7. However those with malaria had mean temperature $0.1\text{ }^{\circ}\text{C}$ more than those without.

Table 7: Children’s Temperature ($^{\circ}\text{C}$) by malaria status

Malaria status	N	Temperature $^{\circ}\text{C}$				
		Mean	SD	Median	Minimum	Maximum
Positive	55	36.9	0.4	36.9	36	37.8
Negative	56	36.8	0.4	36.8	36	38.4
Total	111	36.9	0.4	36.8	36	38.4

4.3.2 Respiratory rate

A summary of respiratory rate by gender showed no difference between males and females. Pooling gender and comparing mean respiratory rate by malaria status, there was no significant mean respiratory rate difference between malaria positives and malaria negatives ($P=0.217$) as shown in Table 10. Malaria positives had 1.3 respirations/min more than malaria negatives (Figure 2)

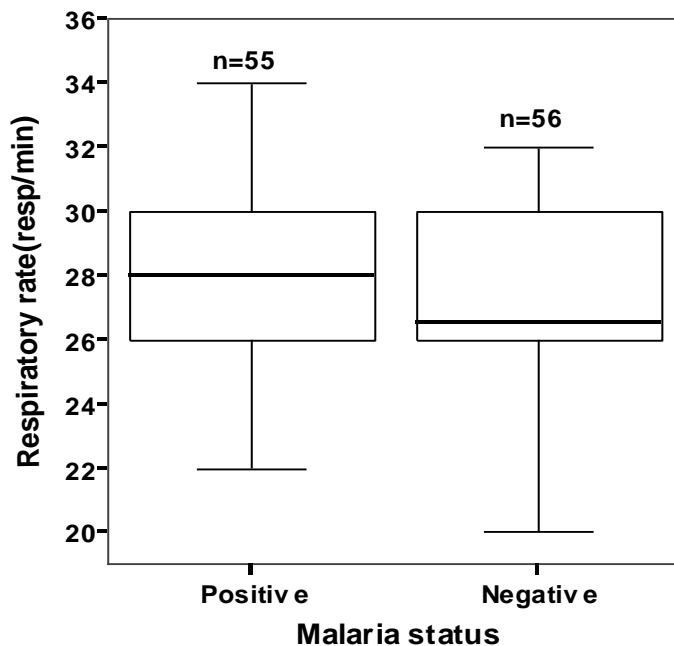


Figure 2: Respiratory rate (respirations/min) by malaria status

4.3.3 Pulse Rate

Pooling gender and comparing mean pulse rate by malaria status, there was no significant difference between malaria positives and malaria negatives ($P=0.089$) as

shown in Table 9. Malaria positives had 2 beats/ minute more than malaria negatives (Table 8).

Table 8: Pulse rate (beats/min) by malaria status

Malaria status	N	Pulse rate (beats / min)				
		Mean	SD	Median	Minimum	Maximum
Positive	55	96.1	4.6	96	86	108
Negative	56	94.3	5.0	94	78	108
Total	111	95.2	4.9	94	78	108

4.3.4 Blood pressure

Sixty-one percent of the children, a majority of whom were malaria positive had diastolic pressure >60 m/Hg (Figure 3). There was no association between blood pressure and malaria status (P=0.244, Odds ratio 1.7 (0.8 – 3.6)) as shown in Table 9.

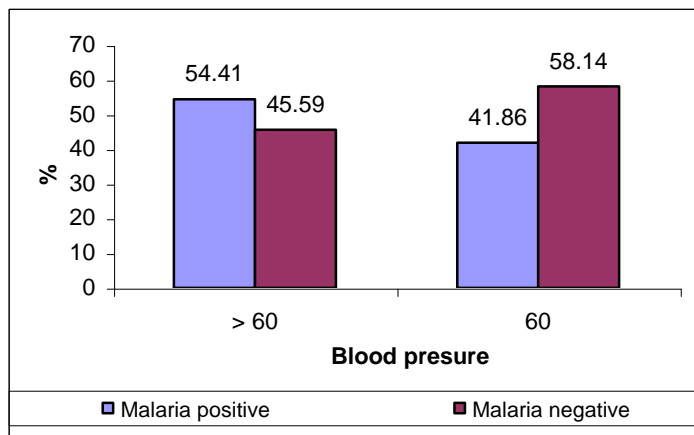


Figure 3: Blood pressure (mm/Hg) by malaria status

Table 9: Clinical profile by malaria status.

Variables/ Categories	Malaria status				Odds Ratio	Lower	Upper	P value
	Positive		Negative					
	N	%	n	%				
Temperature (°C)								
> 37	18	62.1	11	37.9	2.0	0.8	4.7	0.135
<= 37	37	45.1	45	54.9				
Respiratory rate (respirations/min)								
> 25	48	52.7	43	47.3	2.1	0.8	5.7	0.217
<= 25	7	35.0	13	65.0				
Diastolic blood pressure (mm/Hg)								
> 60	37	54.4	31	45.6	1.7	0.8	3.6	0.244
<= 60	18	41.9	25	58.1				
Pulse rate (beats/min)								
> 95	32	58.2	23	41.8	2.0	0.9	4.2	0.089
<= 95	23	41.1	33	58.9				

4.4 Nutrition Status

4.4.1 Mid Upper Arm Circumference (MUAC)

Ninety-six percent of the children were within the normal range of MUAC while 3.6 % were within low range (Table 10).

Table 10: MUAC values by malaria status

MUAC values	Malaria status			
	Positive		Negative	
	N	%	N	%
Normal	53	96.4	54	96.4
Low	2	3.6	2	3.6

Pooling gender and comparing MUAC by malaria status, there was no difference between malaria positives and malaria negatives. Malaria positives and malaria negatives were equal (Figure 4).

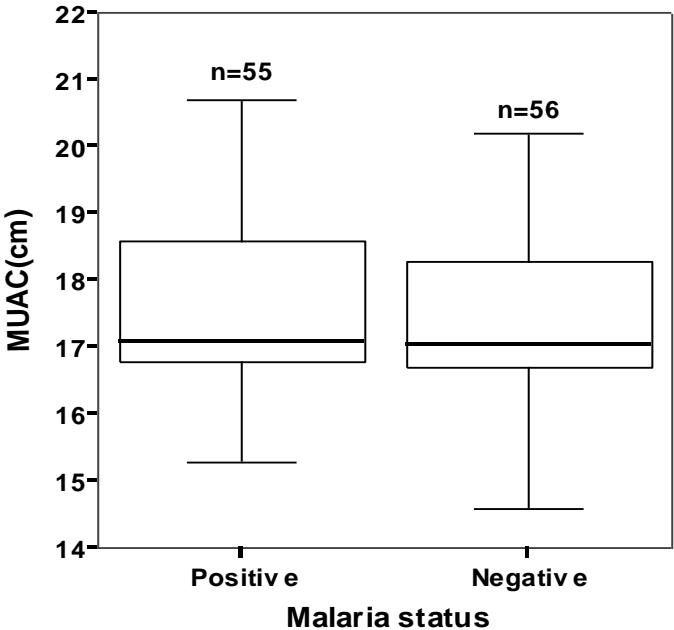


Figure 4: MUAC (cm) by malaria status

4.4.2 Body Mass Index (BMI)

Considering BMI Z scores, 67.6 % of the children were within normal range, 22.5 % mild, 7.2 % moderate while 2.7 % were severe range (Table 11). The difference in BMI between malaria positives and malaria negatives was not significantly different.

Table 11: BMI values by malaria status

BMI Values	Malaria status				Total %
	Positive		Negative		
	N	%	N	%	
Normal	43	78.2	32	57.1	67.6
Mild	9	16.4	16	28.6	22.5
Moderate	2	3.6	6	10.7	7.2
Severe	1	1.8	2	3.6	2.7

4.4.3 Nutrition Z scores by malaria status.

The level of stunting, underweight and wasting in the study population was 9.9 %, 7.2 % and 3.7 % respectively. Distribution of Z scores by malaria status revealed an insignificant association. None of the variables associated significantly with malaria (Table 12).

Table 12: Children Z scores by malaria status

Variables	Malaria status				P value
	Positive		Negative		
	N	%	N	%	
Height-Age-Z scores					
<= -2 (stunted)	6	54.5	5	45.5	0.761
> -2 (Not stunted)	49	49.0	51	51.0	
Weight-Age-Z scores					
<= -2 (Underweight)	2	25.0	6	75.0	0.271
> -2 (Normal weight)	53	51.5	50	48.5	
Weight-Height-Z scores					
<= -2 (Wasted)	1	25.0	3	75.0	0.618
> -2 (Not wasted)	52	50.5	51	49.5	

4.5 Micronutrient status

4.5.1 Haematology profile

Thirty-one percent of the children under study had mild anaemia while 69.4 % were normal. A relationship between haemoglobin and malaria status revealed no association, P=0.221 (Table 13).

Table 13: Haemoglobin levels by malaria status

Haemoglobin level	Malaria status				P value
	Positive		Negative		
	N	%	N	%	
>7g/dl<10g/dl (mild anaemia)	20	58.8	14	41.2	0.221
>10g/dl (Normal)	35	45.5	42	54.5	

Although malaria negatives had higher units of mean WBC, haemoglobin level, MCV, MCH, and MCHC compared to malaria positive children, the difference was not significant (Table 14). However it was noted that malaria negatives had a mean platelet count of 304.8 while malaria positives had a mean platelet count of 257.6, significant (P=0.001).

Table 14: Full Haemogram by malaria status

Variables	Malaria status									
	Positive					Negative				
	N	Mean	SD	Min.	Max.	n	Mean	SD	Min.	Max.
WBC(SI units)	55	7.3	1.8	4.3	15.4	56	7.9	2.4	4.9	17.3
RBC(SI units)	55	4.5	0.4	3.4	5.3	56	4.5	0.3	3.6	5.1
Lymphocytes(SIunits)	55	3.5	1.0	1.0	7.3	56	3.5	0.9	2.2	7.2
Haemoglobin(g/dL)	55	12.5	1.0	10.4	14.9	56	12.6	1.0	10.5	14.8
MCV(fL)	55	82.0	4.6	69.2	89.7	56	82.6	4.8	67.3	91.6
MCH(pg)	55	28.0	1.9	22.5	31.2	56	28.1	2.6	16.1	31.7
MCHC(g/dL)	55	34.2	0.7	32.5	35.6	56	34.3	1.0	32.2	36.9
Platelets(SI units)	55	257.6	78.4	128.0	558.0	56	304.8	66.5	192.0	531.0
MO#	54	0.7	0.6	0.1	2.2	54	0.9	1.1	0.1	6.9
GR#	54	3.2	1.3	1.3	7.7	55	3.6	1.6	1.1	10.4
HCT(SI units)	55	36.5	2.8	30.5	42.2	56	36.7	2.9	30.0	42.5
RDW	55	13.5	1.1	11.4	16.3	56	13.2	0.9	11.6	16.5
MPV	55	8.3	1.0	6.5	11.7	56	8.5	0.9	6.5	11.8
PCT	54	0.2	0.1	0.1	0.4	55	0.3	0.1	0.2	0.4
PDW	55	16.3	0.6	15.1	18.2	56	16.2	0.5	15.3	18.2

4.5.2 Retinol status

Retinol levels varied between 0.108 – 2.301 $\mu\text{mol} / \text{L}$ and they were distributed across three categories (vitamin A deficient, low marginal, and adequate) as shown in Table 15. Mean retinol levels of males and females was similar, $P=0.672$. Pooling gender and comparing mean retinol by malaria status, there was no difference between malaria positives and malaria negatives, $P=0.402$. However malaria positives had 0.071 $\mu\text{mol} / \text{L}$ more than malaria negatives (Figure 5). Missing data of 25 samples was attributable to insufficient serum being available to determine retinol levels. This could not have altered results as the 86 samples tested were more than the calculated sample size.

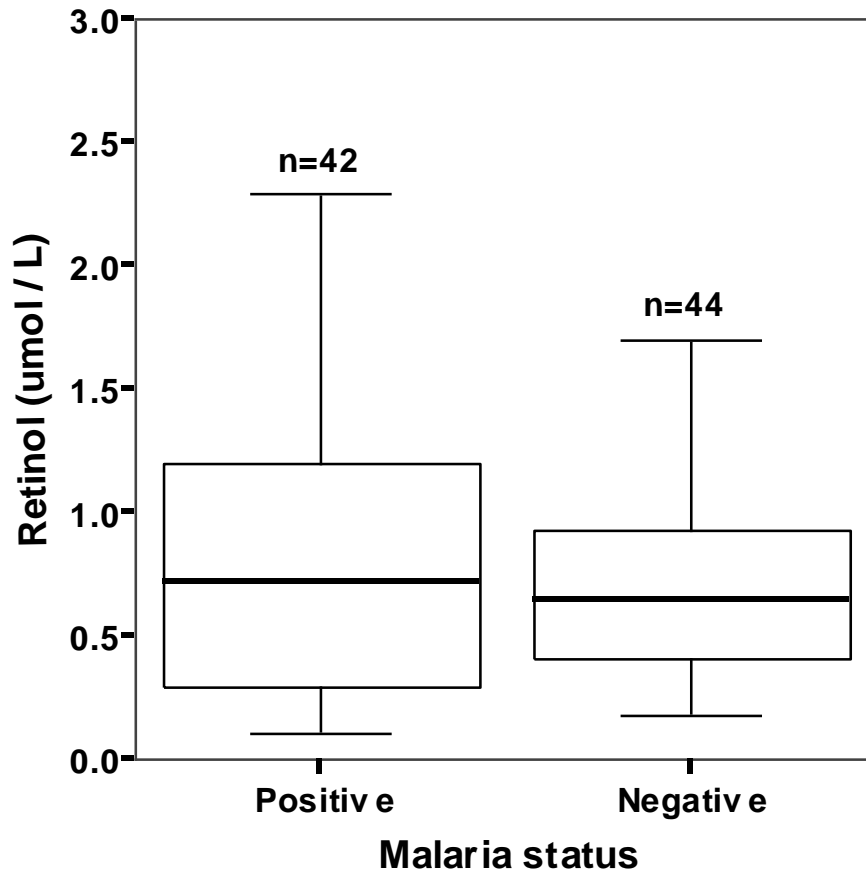


Figure 5: Retinol levels ($\mu\text{mol / L}$) by malaria status

Table 15: Association between serum retinol levels and malaria

Retinol ($\mu\text{mol / L}$)	Malaria status				Odds Ratio (O.R)	95% C.I of O.R		P value
	Positive		Negative			Lower	Upper	
	N	%	N	%				
< 0.7 (Vitamin A deficient)	21	47.7	23	52.3	Reference category			
0.7 - 1.07 (low marginal)	7	35.0	13	65.0	1.7	0.5	5.84	0.497
> 1.07 (adequate)	14	63.6	8	36.4	0.52	0.16	1.68	0.337
Total	42	48.8	44	51.2				

4.5.3 Zinc status

Zinc levels for all the children ranged between 80 – 376 $\mu\text{g} / \text{dL}$, which was higher than the set cut-off point of 65 $\mu\text{g} / \text{dL}$. Mean zinc levels between males and females were not different, $P=0.606$.

Pooling gender and comparing mean zinc by malaria status, there was no difference between malaria positives and malaria negatives, $P=0.762$ (Table 16). Malaria positives were 8 $\mu\text{g} / \text{dL}$ higher than malaria negatives (Figure 6). Missing data in the numbers tested was attributable to insufficient serum being available to determine zinc levels.

Table 16: Mean serum zinc levels ($\mu\text{g} / \text{dL}$) by malaria status

Malaria status	N	Mean	SD	Median	Minimum	Maximum
Positive	35	192.6	70.9	188	80	376
Negative	25	198.5	76.3760	180	97	352
Total	60	195.1	72.5	183	80	376

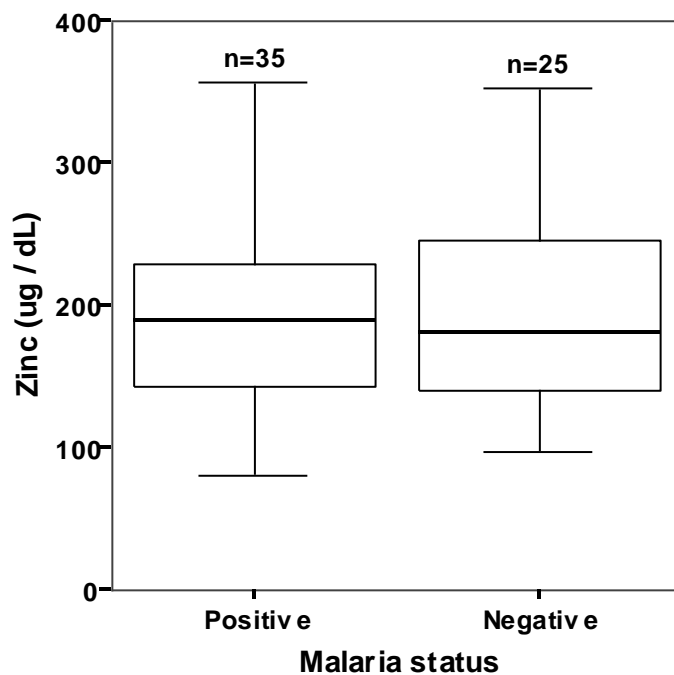


Figure 6: Serum zinc levels ($\mu\text{g} / \text{dL}$) by malaria status

4.5.4 Serum retinol and zinc levels

Serum retinol and zinc levels were graphically examined to assess any relationship. The scatter plot in Figure 7 showed minimal interaction between retinol and zinc. The gradient of the graph was small. The same procedure was repeated considering each gender independently (Figure 8). It was notable that while there was a positive correlation between serum zinc and retinol among males, the relationship was inverse among the females.

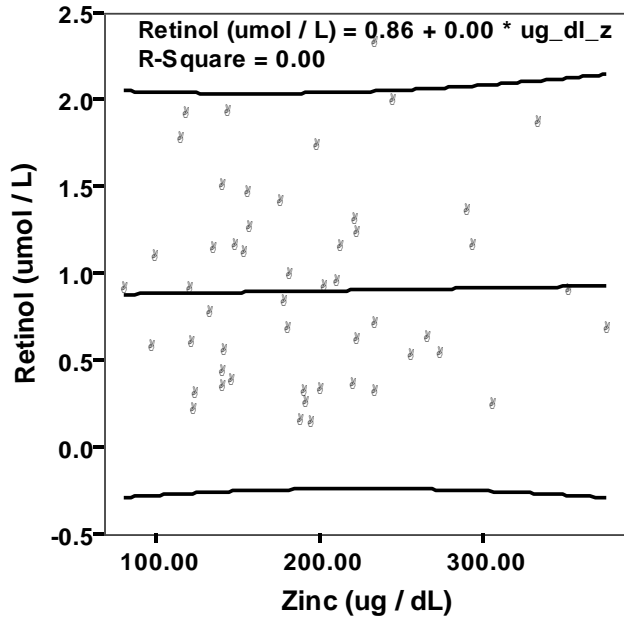


Figure 7: A scatter plot showing the relationship between retinol and zinc.

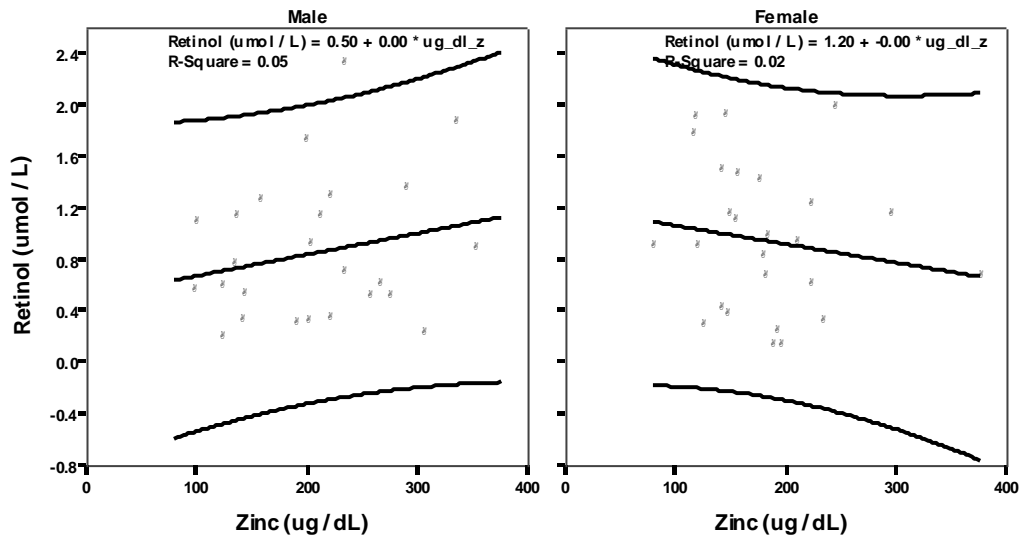


Figure 8: A scatter plot showing the relationship between retinol and zinc by gender.

CHAPTER FIVE

DISCUSSION

5.1 Socio-demographic characteristics and malaria status

Use of mosquito control methods was not associated with reduced prevalence of malaria. Probably the reason for this lies in the use of the control methods as information on their adequacy and maintenance was not covered in the study. In a number of controlled trials across Africa, it was established that ITNs reduced all-cause mortality among children <5 years by approximately 20 % (Lenegler, 2002). However trials were more difficult to measure under field conditions where the main barriers to their effective use included re-treatment with insecticide every six months, availability and cost of insecticide and the netting materials (Lenegler and Snow, 1996).

Seventy-three percent of guardians / parents whose children were malaria positive had never attended school. This corroborates findings of several studies carried out in Asia and the Pacific Region. Improvement in the condition of nightblindness in Bangladesh was accomplished by conducting nutrition education among parents (Yusuf and Islam, 1994). Lack of knowledge is attributable to inadequate access to formal and informal education including nutrition, health, family planning, and vocational training (Gillespie and Mason, 1991). Lack of knowledge about nutrition and hygiene and ability to apply this information to everyday life is an important factor related to health. Even with limitations imposed on nutritional well-being by poverty and food shortages, better use

of available foods can be made by people who know how to utilize them to promote nutritional health.

5.2 Nutritional status of the children and malaria status

There is evidence to support the fact that malaria is causally related to decrease in nutritional status (Shankar, 2000; Snow *et al.*, 1997). What is often debated is whether undernutrition places children at increased or decreased risk for malaria infection (Muller *et al.*, 2003; Fishman *et al.*, 2004). Z-scores of the children under study indicated that about 80 % of them could be classified as normal (healthy). None of the nutrition status indicators associated significantly with malaria parasitaemia. This agrees with findings from prospective studies that found no relationship between baseline nutritional status and subsequent incidence of malaria (Nyakeriga *et al.*, 2004; Muller *et al.*, 2003). Most reviews of this subject conclude that chronic undernutrition likely increases the risk of malaria morbidity and mortality (Caulifield *et al.*, 2004; Fishman *et al.*, 2004).

5.3 Frequency of feeding and malaria status

Frequency of feeding emerged as an important variable on the state of malaria parasitaemia. Children without malaria had significantly higher frequencies of feeding per week compared to those with malaria. However, it should be noted that the data collected gave the dietary pattern of households in general rather than information on specific nutrients. Although the amounts and combination of foods eaten was not taken into consideration, it was noted that the children had fairly diversified diets whereby

95.5 % consumed food items from 4 broad food groups in the 7 days under study. This could explain the fact that these children had higher nutritional status compared to both national and provincial statistics respectively (KDHS, 2008). Although most of the children ate a lot of fruits, vegetables and cereals most likely because they are locally produced, 75 % of them had less than adequate levels of vitamin A. The culprit here could be cooking vegetables with little or no fats and oils which could lead to loss of micronutrients as well as reduce their bioavailability (Ruel, 2002). But even in studies where families consumed adequate amounts of energy (kilocalories) and protein, this did not automatically equate with adequacy in terms of vitamins and minerals (WHO, 2002). Individual nutrients have many specific metabolic functions including primary and supporting roles, no nutrient ever works alone. Intimate metabolic relationships exist among all the basic nutrients and their metabolites. We may separate nutrients for study purposes, but they do not exist that way in the body (Williams, 1994). This could also explain why there were no significant differences of individual nutrients (serum levels of zinc, vitamin A and iron) in both malaria positive and negative children as opposed to wholesome dietary diversity.

Dietary diversity has long been recognized by nutritionists as a key element of high quality diets. Increasing the variety of foods across and within food groups is recommended in most dietary guidelines because it is thought to ensure adequate intake of essential nutrients and to promote good health (WHO, 1996). Lack of diversity may

contribute to the development of specific nutrient deficiencies and may be a factor contributing to increased morbidity and poorer survival in these children (Faber, 2005).

5.4 Clinical signs and malaria status

Although not statistically significant, children with malaria parasitaemia had a temperature of 0.1 °C higher than the controls. Any temperature above 37.5 °C is a sign of physical illness, often the first indication that there is some disturbance of body function. The malaria parasite spends most of its life-cycle in red blood cells, feeds on their haemoglobin and then breaks them apart, causing fever. It is believed that fever actually helps the body to combat infection because disease causing organisms survive less readily, and production of immune bodies increase, when body temperature is raised above normal (Roper *et al.*, 1990). It is however appreciated that apprehension and physical activity are two factors that may affect the child's body temperature. A fever might be found in a school child who is experiencing fear and tension.

The normal rate of breathing at rest decreases with increase in age. At age 5 the normal rate varies from 20-25 respirations/min, while at age 10, the variation is between 17-20 respirations per minute (Roper *et al.*, 1990). May be due to an increase in oxygen requirements in disease, children with malaria parasitaemia had 1.5 respirations/min more than children without, However, it is noted that apprehension, crying, physical activity as well as the examination procedure itself can alter the heart and respiration rate of a child.

Average heart rate for children (6-10 years) at rest is 95 beats/minute. In this study, mean respiratory rate by malaria status was not statistically different although the girls had a minimum rate of 86 against 78 beats/min noted in boys. Marked increases in temperature are accompanied by increments in pulse and respiratory rates because oxygen requirements are known to increase 10 % for every 1 °C rise in temperature (Lowrery, 1978).

BP increases with age, primarily because of the decreased distensibility of the veins. Although there was no significant association between BP and malaria status, 54 % of the children with malaria parasitaemia had a value of >60 mm Hg. Any factor, in this case malaria parasitaemia, that increases peripheral resistance or cardiac output affects the blood pressure (Malasarios *et al.*, 1991).

5.5 Haemogram profile and malaria status

Children diagnosed with mild anaemia (7-10 µg/dL) constituted 30.6 % of the population. None of the children had severe anaemia (< 7 µg/dL). RBC level was the same in both malaria positive and negative children. There is some experimental evidence to suggest that iron binding proteins protect animals from infection by withholding it from the invading organisms that require it for growth (Weinberg, 1984). The impact of malaria on iron metabolism is a combination of both the effects of hemolytic anaemias and that of infections; the outcome can be either an increase or

reduction in serum iron levels. There was a slight reduction in this study as children without malaria had higher mean haemoglobin, MCV, MCH, and MCHC levels compared to children with malaria, although not statistically significant.

Several studies have shown that the assessment of iron status in the presence of malaria is complicated by the modification of most of the routinely used biochemical and hematological indices of iron status (Filteau and Tomkins, 1994). Serum ferritin levels may be lower in infected individuals due to the deposition of iron in body store. Transferrin may be lower because of infection and MCHC could be lower due to an increased ratio of red cell volume in relation to haemoglobin content (Abdalla, 1990).

Children without malaria had a higher mean platelets count than those with malaria ($P=0.001$). Lower platelet count in children with malaria corroborates studies that have shown that platelets contribute to disease pathology in animal and human malaria (Lou *et al.*, 2001). In human infections, platelets may form clumps with infected erythrocytes (Pain *et al.*, 2001). One explanation to this finding could be that low levels of platelets may not only be a marker of parasite burden but may also be protective from severe disease (David *et al.*, 2002).

Although not statistically significant, children without malaria had a higher mean WBC than those with malaria. Malaria is known to be accompanied by an abnormal increase in

number of circulating WBC (leucocytosis), although a decrease (leukopenia) may also occur. Leucocytosis has been associated with severe disease (Molyneux *et al.*, 1989). An increase in large mono nuclear leucocytes (Monocytosis) and increased numbers of circulating lymphocytes are also seen in acute infection, although the significance of these changes is not established [Abdalla, 1988].

The haematology results are in line with findings of a controlled study on haemogram of malaria patients in Calcutta which revealed that haemoglobin levels, haematocrit values, WBC and platelet counts of malaria cases were significantly lower than in the matched controls (Biswas *et al.*, 1999).

5.6 Serum zinc levels and malaria status

Zinc levels for all the children ranged between 80-376 µg/dL. None of the children was deficient of zinc. Inasmuch as this was not the expected outcome, it was in conformity with studies that have shown that infection does not affect the serum zinc levels (Brown *et al.*, 1998). In addition, a randomized trial in Burkina Faso also did not show any difference in malarial illness between two groups that were provided either placebo or supplemented with zinc for 6 days a week for 46 weeks (Shankar *et al.*, 2000).

However, this result was noted to be in contrast with animal and adult data that showed a significant reduction in plasma zinc with acute inflammatory stresses (Beisel, 1977). Serum zinc concentrations vary inversely with malaria parasitaemia and may preferentially protect against more severe malaria with high levels of parasitaemia (Isaksen and Fagerhol, 2001). Deficiency of zinc will therefore be beneficial for malaria cases as reduction of zinc in circulation reduces zinc available for metabolism of micro-organisms during infection creating the same advantage as in iron.

Presence of infection in general, results in sequestration of zinc in the liver (Cousin and Leinart, 1988), and decreased circulating level of zinc, which will reduce the availability of zinc to other tissues. It is not known whether these alterations in zinc metabolism may benefit the host by making more zinc available for particular processes in the liver or by reducing zinc availability in the peripheral blood. Missing data attributed to unavailability of sufficient serum for testing might not have altered much as results of other tested parameters (obtained by assessing the calculated sample size) like serum vitamin A levels, nutrition status and vital signs, were not significantly different among children with and without malaria.

5.7 Serum vitamin A and malaria status

Of the total population under study, 51.2 % had vitamin A deficiency ($< 0.7 \mu\text{mol/L}$), 23.3 % had low marginal vitamin A status ($0.7 > 1.07$), and 25.6 % had adequate levels of vitamin A (> 1.07). Mean serum retinol among children with malaria and those without was not statistically significant. This is consistent with findings of studies on micronutrient levels of children with acute malaria infection.

Fluctuations in vitamin A metabolism during acute infection have been shown (Filteau, 1999). Similar observations were found in malaria infants that were malnourished in Ghana (Filteau *et al.*, 1993). Malaria thus has a variable effect on serum retinol probably due to the fact that metabolic stress is more due to the parasite-immunity relationship than to density of the parasite alone (Adelekan *et al.*, 1997). In contrast, micronutrient deficiency has been associated with increased morbidity and mortality from malaria, and malaria, in turn may contribute to poor nutritional status, reflecting the classic, vicious cycle of malnutrition and infection (Scrimshaw *et al.*, 1968). This is justified by the fact that in the course of infection, nutrients move from circulation to the tissues causing a reduction from circulation (Keusch, 1998). The low levels of vitamin A observed in malaria patients is probably due to the fact that vitamin A is an anti-infective vitamin, which plays an important role in immunity to infectious diseases. Thus during malaria

infection, vitamin A may enhance both antibody-mediated immunity and cell-mediated immunity.

It was notable that while there was a positive correlation between serum zinc and retinol among males, the relationship was negative among females suggesting that boys and girls may stimulate different forms of immunity. Boys are at higher risk than girls, which may reflect either physiological differences or different cultural practices in rearing children (Demaeyer, 1986).

5.8 Study Limitations

The severity of parasite density was not assessed. This may have had a bearing on micronutrient levels of these children.

Since this study was school-based, progression of micronutrient levels to symptomatic malaria were not known.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Use of mosquito control methods was not associated with reduced prevalence of malaria.

There was a significant association between malaria status and guardian/ parent level of education ($P=0.018$).

None of the nutrition status indicators associated significantly with malaria parasitaemia.

Children with higher nutritional intake were more likely to test negative for malaria parasitaemia. This suggests that high levels of the nutrients are critical in lowering susceptibility to malaria.

There was no significant mean difference in temperature, blood pressure, and respiration rates between children with and without malaria. However those with malaria had remarkably higher mean levels than those without.

Although not statistically significant, children without malaria had higher mean haemoglobin, MCV, MCH, and MCHC levels compared to children with malaria.

Serum zinc, vitamin A, and iron levels showed no significant mean differences between children with/without malaria parasitaemia.

Literature is not unanimous on the link between these micronutrients and the different phases of malaria. Probably more research with a larger sample size might clarify issues.

6.2 Recommendations

Most findings cited in this study are from studies done during the acute phase of malaria infection and subsequent morbidity and mortality. More longitudinal studies should be done in the asymptomatic parasitaemia phase to confirm causality.

Prevalence of asymptomatic malaria was about 5 % in the study population. This suggests that presumptive treatment of asymptomatic parasitaemia would be an efficient means of preventing malaria.

Parents/guardians should be empowered with nutrition and health education programmes.

It is recommended that nutrition components (especially high feeding frequency and dietary diversity) be integrated into existing malaria intervention programs.

The relationship between malaria parasitaemia and nutrition of children is complex and requires additional research.

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APPENDICES

APPENDIX 1: KEMRI-ERC ETHICAL APPROVAL COPY



KENYA MEDICAL RESEARCH INSTITUTE

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KEMRI/RES/7/3/1

29th May 2006

Ms. I. Mogiti Makori,
CPHR,
NAIROBI

Thro'
The Director,
CPHR,
NAIROBI

Dear Madam,

RE: SSC Protocol No. 1014 (Revised) – Serum Vitamin A and Zinc and susceptibility to infection with malaria among school children in Kisii District

*Forwarded with Compliments
to 8/6/06.*

We refer to your letter dated March 20th 2006 and acknowledge receipt of the revised protocol.

We note that the selection criteria have been modified; all samples will be handled within the KEMRI laboratories; and that this will be a case-control study to assess the vulnerability of an individual to malaria infection within the same ecological zone. The Committee is satisfied that the issues raised at the 128th meeting of the KEMRI/National Ethical Review Committee have been adequately addressed.

The protocol is hereby granted approval for you to embark on your study. You are however, responsible for reporting to the Ethical Review Committee any changes to the protocol or in the Informed Consent Document. This includes changes to research design or procedures that could introduce new or more than minimum risk to human subjects.

C.L.W
C. L. Wasunna,
For: Secretary,
KEMRI/NATIONAL ETHICAL REVIEW COMMITTEE

APPENDIX 2: STUDY INFORMATION SHEET

The following information is to enable you to give voluntary, informed consent to your child's participation in this study. Please read the information carefully before signing the consent form. **You and your child's participation are voluntary.**

The information contained herein is to be read and questions answered in a language that the parent/guardian is fluent in (English, Kiswahili or vernacular) before signing the consent form.

Title of study: Serum retinol and zinc and susceptibility to infection with malaria among school children in Kisii District.

Investigators name: Irene Mogiti Makori

Address: ITROMID- KEMRI

Aim of study: The purpose of this study is to investigate how serum levels of vitamin A and zinc relate to susceptibility to malaria. The findings will be used in nutritional interventions aimed at raising the children's immunity by improving their nutritional status.

What the study involves

1. An experienced laboratory technician will take some venous blood from your child for testing. A few drops of the drawn blood will be tested for malaria and the rest will be taken for testing of serum levels of vitamin A and zinc. If your child has malaria parasite, you will be requested to take him/her for treatment. Taking this blood sample may cause a bit of discomfort to your child but will not cause any harm. We will use sterile disposable needles.
2. A trained field assistant will take your child's temperature and measure the weight, height and mid-upper arm circumference. The figures obtained will be combined with the results obtained from the blood tests.
3. The investigator will request you to complete a structured questionnaire inquiring about dietary habits of your child, use of anti-vector measures to prevent malaria, recent use of anti-malarial drugs, and a significant medical history. You are free to choose questions to answer.

Confidentiality

All the information we get from you and your child will remain confidential. Nobody outside the study team will have access to the information. Your name and child's name will not appear on any report that will be produced.

APPENDIX 3: STUDY CONSENT FORM

Consent form (completed by all participants' parents)

You are requested to read the information sheet carefully or it is explained clearly to you before signing this consent form. If there are any questions about the study, ask the investigator before signing the consent form.

**I HAVE READ THE INFORMATION SHEET CONCERNING THIS STUDY
AND I UNDERSTAND WHAT WILL BE REQUIRED OF MY CHILD IF
SHE/HE TAKES PART IN THE STUDY.**

I AGREE TO TAKE PART IN THE STUDY

Name: _____

Signed (or thumb print) _____

Date: _____

APPENDIX 4: VITAMIN A AND ZINC STUDY

QUESTIONNAIRE

Form serial Number

--	--	--	--	--

Interviewers' initials

--	--	--

Date of interview

--	--	--

Day / Month / Year

District _____ Location _____

School _____ Class _____

Guardian's/ Parent's name _____

Child's name _____ sex of child: Female Male

Date of birth

--	--	--	--

 Age in years _____
Day / Month / Year

Guardians/Parents name _____ Occupation of Parent/Guardian _____

Level of education of Mother/Guardian None Primary Secondary College

Schooling and current Health status

1. How long has the child been in this school _____ Years _____ Months _____

2. Has your child been absent from school due to illness during this last one month?

Yes No

3. Is your child ill today? Yes No

If yes, what is the problem?

4. Does your child use any mosquito control measures? Yes No

If yes, control measures used _____

Medical History

5. Has your child been on any medication during the last one-month? Yes No

If yes, what was she/he treated for? _____

6. Has the child ever had any blood transfusion? Yes No

If yes, how long ago was the last transfusion given?

2 weeks a month 2months

Other (specify) _____

Vital signs

7. Auxiliary temperature ____ °C
8. Respiratory rate _____ respirations/min
9. Blood pressure _____ mmHg
10. Pulse rate/heart rate ____ beats/min

Anthropometric measurements

11. Weight _____ Kg
12. Height _____ cm
13. MUAC _____ cm

Lab results

14. Malaria parasites Positive Negative
15. Haemoglobin level _____ g/dL
16. Serum Zinc _____ µg/dL
17. Serum Vitamin A _____ µmol/L

APPENDIX 5: FOOD FREQUENCY QUESTIONNAIRE

How many times per week are the following foods consumed at your home?

	Food types	No. of times per week		Food Types	No. of times per week
Cereals	Maize Millet Rice Wheat		Fruits	Sweet bananas Pawpaws Mangoes Bananas (ripe) Pineapples Oranges Passion Guavas	
Animal products	Meat Fish Chicken Milk Eggs Liver Matumbo		Tubers/Plantain	Sweet potatoes Cassava English potatoes Arrow roots Raw bananas	
Pulses	Beans Green grams/dengu Peas Groundnuts		Vegetables	Sukumawiki Cabbage Managu Chinsaga/nights hade Kunde Pumpkin leaves Emboga	