THE ROLE OF MALARIA RAPID DIAGNOSTIC TEST ON THE MANAGEMENT OF MALARIA AMONGST CHILDREN IN KISARAWE DISTRICT, COAST REGION

By

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CERTIFICATION

The undersigned ce rtify they have read and hereby recommend for acceptance a dissertation entitled ***The Role of Malaria Rapid Diagnostic Test on the Management of Malaria amongst children in Kisarawe District, Coast Region 2009,*** in fulfillment of the requirements for the degree of Master of Science in Epidemiology and Laboratory Management of the Muhimbili University of Health and Allied Sciences.

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I, Stanley Silas Lyimo, declare that this **dissertation/thesis** is my own original work and that it has not been presented and will not be presented to any other University for a similar or any other degree award.

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DEDICATION

This work is dedicated to:

My parents - Silas and Agnes Lyimo

My beloved wife Irene and my children Emmanuel, Silva, Kelvin and my late son Jensen whose memories remain infinitely

ABSTRACT

Malaria is a disease caused by protozoa parasite of the genus *Plasmodium* and infection occurs, following bite of infected female *Anopheline* mosquito. Malaria diagnosis continues to be a major challenge in malaria control strategies in Sub–Sahara Countries including Tanzania. Giemsa staining technical is widely practiced but mis-diagnosis has coursed troubles. The objective of this study was to determine the role of malaria rapid diagnostic test (mRDT) in routine clinical practice for the management of malaria episode amongst children.

Methodology

This was a cross-sectional study involving children aged five years and below attending a health care facility due to fever. A convenient sampling method was used to enroll children and blood samples were collected for mRDT, and smear microscopy. Exit interview was done. The gathered data was analyzed using Epi info soft ware, version 3.5.1.

Results

Results revealed a Sensitivity of RDT of 86% and Specificity of 98% and Predictive Value Positive 97% when the gold standard is reader two. For parasite density the linear Coefficient Correlation of two readers was 0.86, hence the linear correlation coefficient = 0.92 which is within normal acceptable range. There was discordant in parasite prevalence of which reader two had 17.2%, and reader one had 13.8%, an indication of misdiagnosis of reader one. However, even though 66 (22.7%) of children were treated based on mRDT only 50 (17.2%) should have been treated so 16 (5.5%) of children were given ALU unnecessarily.

Discussion

Malaria rapid diagnostic test is simple to perform and we can rely on the test because of the sensitivity and specificity that it has in detecting the presence of malaria parasites in

blood. The test detects the presence of parasite antigen (HRP-2) so the chances that diagnosis can fail are less.

Conclusion

From this study, we recommend the roll out of the mRDT especially to areas with poor infrastructure. We recommend investigation of all children with fever using RDT before treatment and whenever facility allows, all the positive cases to be confirmed with microscopy. However, in situation where microscopy is not possible, all RDT positive cases should receive treatment.

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LIST OF ACRONYMS AND ABBREVIATIONS

ACT	Artemesinin Combination Therapy
Alu	Artemether/lumefantrine
AO	Acridine Orange
ARI	Acute Respiratory Infection
CT	Combination Therapy
DNA	Deoxyribonucleic Acid
DED	District Executive Director
DMO	District Medical Officer
GDP	Gross Domestic Product
HRP2	Histidine Rich Protein antigen
IMCI	Integrated Management of Childhood Illness
ITN's	Insecticide Treated Nets
mRDT	Malaria Rapid Diagnostic Test
MOHSW	Ministry of Health and Social Welfare
MUHAS	Muhimbili University of Health and Allied Sciences
NMCP	National Malaria Control Program
NGO	Non Government Organization
PCR	Polymerase Chain Reaction
RBM	Roll Back Malaria
RDT	Rapid Diagnostic Test
SP	Sulfadoxine/Pyrimethamine
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Malaria is a disease caused by protozoa parasite of the genus *Plasmodium* which invades the red blood cells of human, following injection of sporozoites, the infective stage of the parasite to the human blood circulatory system through bite of infected female *Anopheline* mosquito. Four species of malaria parasites known to infect human are, *Plasmodium vivax* (benign tertian), *Plasmodium ovale* (benign tertian), *Plasmodium malariae* (quartan,) and *Plasmodium falciparum* (malignant). Female *Anopheles* mosquitoes transmit malaria parasites during a blood meal when they pick up gametocytes that undergo sexual reproduction in mosquito, generating sporozoites that are injected to human host during next blood meal (Gilles, 1993).

In Africa *P. falciparum* predominates, as it does in Papua New-Guinea and Haiti whereas *P. vivax* is more common in Central and parts of South America, North Africa, the Middle-East and the Indian Sub-Continent. *P. vivax* is rare in sub-Sahara Africa, whereas *P. ovale* is rare outside West Africa. *P. malariae* is found in most areas, but is relatively uncommon outside Africa (Nicholas, 2001).

In sub- Saharan Africa, *Plasmodium falciparum* is a common species and attributes to 13% of malaria specific mortality and 10 – 13 % of maternal deaths (Salim *et al.* 2007). Of the four malaria species pathogenic to humans, *Plasmodium falciparum* causes the most severe infections and is responsible for more than 95% of malaria deaths worldwide (Samba, 2001). These parasites produces sticky secretions which clump together, the infected red blood cells in the circulation, forming clots which sometimes block the fine blood capillaries of the brain, hence causing cerebral malaria mostly in children. It takes up to 5 days from the time the sporozoites enter the body until the first symptoms are manifested, a period referred to as incubation period.

Malaria continues to be a major global health problem, with over 40% of world's population exposed to varying degrees of malaria risk in some 100 countries. There are 300-500 million new cases of malaria every year, resulting in 1.5 to 2.7 million deaths world wide (Goodman, 2000; Samba, 2001).

Malaria is found throughout the tropics because of the optimal weather conditions that favorers the breeding and survival of *Anopheles* mosquitoes of the species *gambiae, funestus, arabiensis* and *bwambwai.* Malaria prevalence's are seasonal, meaning that the pick prevalence's are more observed during rain seasons, when the most efficient vector, the *Anopheles gambiae* are abundant in population (Shif *et al*, 1995).

In Tanzania malaria is the leading cause of outpatient and inpatient health service attendance and the leading cause of death in both children and adults. It contributes to between 40% and 50% of outpatient department attendance, 30% and 40% of bed occupancy in hospitalized patients and about 13% mortality in children bellow five years of age (NMCP, 2002 – 2004).

Children below 24 months experience at least 4-5 life-threatening episode of malaria and about 60% of maternal spontaneous abortions are reported (WHO, 2000). The estimated number of malaria cases per year is 14 – 19 million and the estimated number of deaths in children bellow five years is 80,000 annually (NMCP, 2009). Young children are more susceptible to malaria as they have not yet developed immunity to the parasites (D'Alessandro *et al,* 1995).

In 2004 malaria diagnosis in children under five years of age accounted for 43% of all outpatient attendances and 40% of deaths were recorded in health facilities in Tanzania (NMCP, Annual Report 2004). In Sub- Saharan Africa close to 45% of pregnant women have malaria infection during pregnancy and almost half of women who are pregnant for the first time (primigravidae) will be parasitaemic on their first antenatal visit (Steketee, 1996).

Pregnant women have a high risk of peripheral parasitaemia and placental malaria because their immunity to the parasites is suppressed during pregnancy and the parasites often infect the placenta leading to maternal anemia, still birth, spontaneous abortions and low birth weight. They commonly present with the features like high fever, hyper-parasitaemia, low blood sugar, severe hemolytic anemia, cerebral malaria and pulmonary edema (Schulman *et al*, 2003; Menendeze, 1995).

The most common clinical feature of malaria is fever and the onset of symptoms may resemble flu-like illness. The fever pattern of the first few days of infection resembles that in early stages of many other illnesses caused by bacteria, viruses and parasites (David, 2004).

In infants the early symptoms of malaria may be quite variable and difficult to recognize. They may be limited to poor appetite, restlessness and loss of normal interest in the surroundings. Some children may present with a cough or vomiting and diarrhea (National Guidelines for Malaria diagnosis and Treatment, 2006).

The features of uncomplicated malaria include, fever, headache, joint pains, malaise, body weakness, vomiting, diarrhea, chest pain, poor appetite, anemia and hepato-splenomegaly especially in children (National Guidelines for Malaria diagnosis and Treatment, 2006).

In Tanzania the commonest presentation of severe malaria are severe anemia and cerebral malaria. Other features of severe malaria are convulsions, respiratory distress, bleeding tendency, jaundice, circulatory collapse and acute renal failure (National Guidelines for Malaria diagnosis and Treatment, 2006). Anemia and splenomegaly often develop after some days. Prompt treatment of *falciparum* malaria is essential, even in mild cases, since irreversible complications may rapidly appear (National Malaria Control Report, 2009). There is evidence of decreased malaria transmission in sub Saharan Africa, as well as declining proportion of fevers due to malaria where malaria control interventions has been implemented on a large scale. Recent studies provide

evidence that malaria burden is significantly decreasing both mainland Tanzania and in Zanzibar (Bhattarai *et al,* 2007).

Demographic and health survey done in Dar-es-salaam, Tanzania showed steep reductions in infant mortality due to malaria (Marco, 2004 -2005). This reduction is also observed in areas with previously high levels of transmission (WHO, 2008). A sharp decline of *Plasmodium falciparum* prevalence rates in sub-Saharan Africa from 37% in 1999 to 17 % in 2007 is clearly documented by Guera, (2008).

Constraints of malaria control includes drug and insecticide resistance, weak health systems and infrastructure including human resource capacity, inadequate surveillance, lack of accurate diagnosis leading to over treatment, and efficient abundance of *Anopheles gambiae* (Makundi *et al*, 2007).
Following the wide spread of resistance to chloroquine, Tanzania changed its malaria treatment policy from Chloroquine to Sulfadoxine/Pyrimethamine (SP) monotherapy as the first line drug for the treatment of acute malaria episodes in august 2001. However, over the last 4 years resistance to SP has already been reported to be on the increase.

The studies done in Tanzania (2004) indicate that the mean SP treatment failure is now 25.5% and the mean treatment failure of Amodiaquine, the second line anti-malarial, is 12% (National guidelines for malaria diagnosis and treatment, 2006). In addition, molecular markers of SP resistance have recorded high levels of mutation *(NMCP,* 2007).

The economic impact of malaria on Tanzania's economy shows that malaria consumes an estimated US dollars 119 million of national resources in a year (3.4% of GDP) through direct expenditures of state and households, loss of economic productivity and loss of economic opportunities especially for industrial development (Information Kit, NMCP, 2009).

Presumptive anti-malarial treatment for any fever with obvious alternative cause is widely practiced and studies suggest that this leads to significant over use of anti-malarial drugs (Wang *et al*, 2006).

1.1.1 Treatment of uncomplicated malaria

All fever cases diagnosed as malaria by RDT or microscopy should promptly be given effective treatment. The treatment of *P. falciparum* malaria is based on areas identified as chloroquine resistant. Artemisinin Combination Therapy (ACT) should be given in resistant areas whereas chloroquine can be used in sensitive areas. ACT should be given only to confirmed *P. falciparum* cases found positive by microscopy or RDT.

ACT consists of an artemisinin derivative combined with a long acting anti-malarial (amodiaquine, lumefantrine, mefloquine or sulfadoxine-pyrimethamine). According to current WHO guidelines, ACTs can be given in the second and third trimester of pregnancy. The recommended treatment in the first trimester of pregnancy is quinine.

1.1.2 Treatment based on clinical criteria without laboratory confirmation

All efforts should be made to diagnose malaria either by microscopy or RDT. However, special circumstances should be addressed as mentioned below. If RDT is negative and a microscopy results can not be obtained but cases show signs and symptoms of malaria without any other obvious cause for fever should be considered as 'clinical malaria' and treated (WHO, 2010).

Combination therapy with anti-malarial drugs is the simultaneous use of two or more blood schizonticidal drugs with independent modes of action and different biochemical targets in the parasite. Combination therapy can be either fixed-combination medicinal products, in which the components are co-formulated in the same tablet or capsule, or multiple-drug therapy, in which the components are co-administered in separate tablets or capsules.

Artemisinin based combination therapy (ACT) is anti-malarial combination therapy with artemisinin derivatives as one component of the combination. Artemisinin based combinations are highly efficacious and have the potential to delay the spread of drug resistance (National guideline for malaria diagnosis and treatment, 2006).

Specifically, a combination of Artemether-lumefantrine (Alu) is recommended as first line therapy for uncomplicated malaria while Quinine is recommended as the second line drug in case of treatment failure or the presence of a contraindication to the first line drug for treatment of uncomplicated malaria. Quinine also remains the drug of choice for the treatment of severe malaria (National guideline for malaria diagnosis and treatment, 2006).

Artemether/lumefantrine (Alu) is an oral fixed combination tablet of 20mg Artemether a derivative of artemisinin and 120mg Lumefantrine. This drug combination is effective against all human malaria parasite species. It is highly efficacious against multi-drug resistant malaria parasites with clearance of the parasites from blood within two days. The dosage of Artemether/Lumefantrine is 1.5/12 mg/kg body weight twice a day for three days (National guideline for malaria diagnosis and treatment, 2006).

In children under five years of age, it is recommended that diagnosis and treatment of uncomplicated malaria should be done within 24 hours from the onset of symptoms. Children under five years of age with complicated malaria are treated freely with Artemether injected intramuscularly, as per IMCI (National guideline for malaria diagnosis and treatment, 2006).

In high transmission areas, a significant proportion of individuals have parasitaemia but do not necessarily suffer from malaria disease. An individual with malaria infection may be completely asymptomatic. It should be noted that the aim of diagnosis is to assist in the treatment of the disease and not the asymptomatic infection. Misdiagnosis of malaria is costly and results in considerable mortality because it contributes both to delay in

treatment of the correct diagnosis and to increasing anti-malarial drug pressure and thus resistance, thereby speeding up the obsolescence of affordable drug (Bell *et al,* 2006).

Investigation may be done depending on the capacity of the laboratory and the clinical indications. Laboratory tests should be interpreted in conjunction with clinical findings. The main consequence of over-diagnosis is failure to treat other causes of life-threatening disease. Despite these concerns, presumptive treatment is endorsed because light microscopy which for decades has been the standard for malaria diagnosis, remains inaccessible to most patients because of laboratory infrastructure and technical expertise is required.

1.1.3 Malaria Diagnosis

Malaria is the most common clinical diagnosis made in most countries in Africa but the accuracy of clinical diagnosis is limited by the low specificity of symptoms and signs of malaria. Microscopic examination of Giemsa stained blood smears and the newly developed rapid diagnostic tests are important for malaria diagnosis. Close supervision and quality assurance is required for both approaches (National guideline for malaria diagnosis and treatment, 2006).

In order to minimize the irrational use of anti-malarial drugs and reduce the potential for developing parasite resistance and provide quality services, it is important to improve the accuracy of malaria diagnosis. The accuracy of malaria diagnosis has received renewed interest in recent years due to changes in treatment policies in favor of relatively high cost artemisinin based combination therapies. The use of rapid diagnostic tests based on Histidine-Rich Protein-2 (HRP-2) synthesized by *Plasmodium falciparum* has been widely advocated to save costs and minimize inappropriate treatment of non-malaria febrile illness. This technique is based on detection of malaria antigen. Histidine Rich Protein II is a water soluble antigen produced by trophozoites and young gametocytes and parasite lactate dehydrogenase produced by live trophozoites and gametocytes from intact erythrocytes infected with ring form trophozoites, schizonts and immature gametocytes (Haword *et al,* 2000).

Rapid diagnostic tests are qualitative techniques based on the detection of malaria parasite antigens. Rapid diagnostic test is an Immune-chromatography test based on the capture of the parasite antigens from the blood. It detects malaria antigen in a small amount of blood, usually 5-15 µl.

The tests can be done by minimally trained personnel and the rapid (can be performed in about 15 minutes) and are highly sensitive and specific with a high diagnostic reliability (Mboera *et al,* 2005). The test may remain positive for up to one month after treatment even though the patient has been cured. Quantification of parasites is not possible hence the tests cannot be used for evaluation of clinical outcome.

Rapid diagnostic tests can be used at all levels; however, it will be preferable to deploy at levels where microscopy is not possible (e.g. dispensary, health centre). Histidine Rich-Protein-2 based RDT are highly sensitive and stable and they have considerable potential as tools to improve the diagnosis of malaria hence allow improved fever management.

SD BIOLINE malaria antigen is one of many RDT commercially available kit which is intended for the detection of malaria infection in human blood sample, indicating differential diagnosis between *Plasmodium falciparam* histidine rich protein-2 uniquely synthesized by *Plasmodium falciparum* and other Plasmodium species (Plasmodium Lactate dehydrogenase (pan, pLDH). This kit is intended for professional use, only for an initial screening test and reactive samples should be confirmed by a supplemental assay such as microscopic examination of blood smear (SD bio-line one step Malaria Antigen Rapid Test Manual, 2009). Results from field studies indicate sensitivity and specificity of around 90% (Dietze *et al*, 1995).

1.2 Problem Statement

Currently the National Malaria Control Program (NMCP) in Tanzania is recommending the introduction of RDT for fever management in routine clinical practice. Initially the introduction is in three regions and this will be rolled out in due time to cover the whole

country. This diagnostic approach is funded by the global health fund. To achieve this objective the NMCP has developed policy guidelines and training manuals. However, the introduction of this new initiative is based purely on research findings that RDT is acceptably sensitive, specific and can be used in remote areas. Data is missing regarding RDT use in management of fever episode in routine practice and how the results will be used to target anti-malarial treatment. This will thus revolve around acceptability of the result by the health care providers. This information will contribute towards the success of deploying malaria rapid diagnostic test on a larger scale.

1.3 Rationale of the study

Tanzania is in the process of up scaling malaria rapid diagnostic test to improve malaria case management. This study is therefore timely to produce results that may have an impact on the implementation. Malaria rapid diagnostic test initiative of appropriately implement will reduce ant malarial use. This leads to rational use of anti-malaria and will delay emergence of drug resistance. SD bio-line has been registered for use in Tanzania. This study also validates the performance of this test. This work is part of the postgraduate requirement.

1.4 Study Objectives:

1.4.1 Broad Objective

To determine the role of malaria RDT on the management of malaria among children of five years and below in Kisarawe district.

1.4.2 Specific Objectives

- To determine the proportion of children with a presumptive malaria diagnosis who are confirmed to have malaria by RDT.
- To determine the validity of malaria RDT for malaria diagnosis in children, using Giemsa technique as gold standard.
- To determine prescribers responses to RDT results.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Laboratory Diagnosis of Malaria.

The malaria parasite was first observed inside red blood cells through a microscope in a blood film by French military doctor Alphonse Laveran on November 6, 1880 in Algeria. By 1902 Giemsa staining and thin films were introduced (Keisser, 2002)

Although not explicitly recorded, the first time that the malaria parasite was ever observed in Tanzania (then Tanganyika) was around 1898 when Robert Koch laid down the first ever plan for malaria control in the country (Beck, 1977). The plan was based on careful examination of blood films, that if positive, required patients to receive quinine. Then, as now, Koch struggled with the ability of health workers to correctly examine blood films under a microscope.

Currently, there are three main approaches to laboratory-based diagnosis of malaria in Tanzania which are Giemsa stain microscopy, Acridine Orange (AO) microscopy and rapid diagnostic tests or RDT. Giemsa stain microscopy requires highly skilled laboratory personnel and a parallel quality assurance system so as to minimize false results AO microscopy is a more complex method that is used in more sophisticated level facilities. RDT have only been recently introduced operationally in Zanzibar in 2007 and they are still being tested operationally in mainland Tanzania.

The first RDT malaria diagnostic devices were introduced in the early 1990s (Schiff *et al,* 1993). This diagnostic devise showed great promise even in the hands of lay community health workers (Premji *et al,* 1994).

The malaria control program of Tanzania and Zanzibar rely on the use of clinical signs to diagnose malaria in children under five and in children over five and adult populations. This approach included in the Integrated Management of Childhood Illness

(IMCI) algorithm for children under five and in specific malaria case management algorithms for all others, has served well until very recently. However, the prevalence on malaria in Tanzania is rapidly decreasing, so it requires a review of current malaria case management policies and practices (both in mainland Tanzania and Zanzibar are currently reviewing their case management algorithms). In addition to the changing prevalence of malaria as justification for changes in case management, more attention is being paid to the need of correctly assess and manage cases of fever that are not malaria.

Misdiagnosis of fever, in any age, but particularly in children under five has been documented as causing increased mortality in groups who did not receive proper treatment for their underlying illness. In mainland Tanzania one study found that 39% of children attending a health facility in a low transmission area were misdiagnosed to as having malaria (Massaga *et al,* 1999). Another study found that only 46% of people treated for malaria had a positive blood film (Reyburn, 2004). Misdiagnosis of fever also includes those cases in which malaria is present in association with another illness but no effort is made to diagnose the co-infection (Makani, 2003). Unfortunately, health workers tend to ignore additional diagnosis once malaria has become the diagnosis. In a study in Uganda, 37% of patient diagnosed malaria also had undiagnosed pneumonia (Kallander *et al*, 2004).

The prompt and accurate diagnosis of any malady is fundamental in the secondary prevention of disease. Clinical diagnosis is best as it is the fastest, easiest and cheapest way to diagnose illness. Of course, it is dependent on several factors, not the least of which is health worker knowledge and skills. For example, children under five suffering from a fever in an area with high prevalence of malaria stand a good chance of being diagnosed accurately with malaria if the clinical protocol calls for classifying as malaria any fever in the under five cohort. However, when clinical diagnosis is not possible, then relying on laboratory-based tests to obtain a diagnosis is the next best thing.

Today, there are many ways to diagnose malaria with laboratory tests, from observation through a microscope of Giemsa-stained parasites which after a hundred years still

remains the gold standard to highly sophisticated molecular methods to fluorescent microscopy. All have advantages and disadvantages for practical use in developing countries' health facilities. The list of characteristics of the ideal malaria test for deployment in developing countries is long and almost impossible to fulfill. Authors describe the ideal test as one that is simple, fast, precise, minimally invasive, require small blood sample, with high sensitivity and specificity, easy to perform and interpret, allows for species differentiation, is both quantitative and qualitative, stable at high temperatures, easily transportable and monitored, easily disposed of once used, requires no electricity, amenable to batch processing, easily labeled, able to differentiate between asexual and sexual forms, and all of this at an affordable price (Murray, 2003).

The Giemsa procedure is deceptively simple but in fact requires a high degree of skill is labor intensive and time consuming. A small amount of blood is taken from a finger prick. This drop is placed on a microscope slide to make thick or thin film. Thin films is made by spreading a drop of blood across the slide using another clean slide while thick films are made by just emulsifying a drop of blood on a slide to size of a coin.

Often both films are done on one slide. The thin film is then allowed to dry and fixed with methanol while thick films are de-hemoglobinized with water. Both are then stained with Giemsa. Thick films allow the examination of a greater volume of blood increasing the sensitivity of the test. Thin films preserve parasite morphology better and are used to quantify and identify parasites (Institute of Medicine, Malaria, 1991).

Although a good microscopist may be able to detect as few as 5– 10 parasites per µl a more generally accepted number is 100 parasites per µl (WHO/CDS/RBM, 2000).

Thick films are examined for parasitized red blood cells in relation to a predetermined number of white blood cells. To determine the parasite density, several microscopy fields are to be observed and all forms of the parasites, the asexual and sexual stages are counted as per parasitized red cells versus 200 white blood cells. The number of parasite

counted per 200 white blood cells is multiplied by 40 to get the number of parasites per micro liter of blood.

Results are usually available in 45 minutes – 1 hour. Under ideal conditions microscopy is excellent for determining whether a patient has malaria or not. However, conditions in developing countries are far from ideal. Microscopes, if not maintained can break down and need repair, reagents need to be supplied regularly, staff needs to be trained, and quality control and supervision are essential. Microscopy accuracy under real life conditions in developing countries can be only 70 – 75% (Amexo, 2004).

In mainland Tanzania, microscopy of thin films has been in use since German colonial times and it is still in use today. It has been a policy since the start of malaria control in Tanzania to examine blood films of feverish patients whenever possible. Current case management policy calls for taking confirmatory tests depending on level of facility.

According to the Tanzania Service Provision Assessment Survey 2006 only one third of health facilities had the laboratory capacity, microscope and supplies to diagnose malaria from blood films (Macros, 2005).

Moreover, malaria laboratory capacity is in inverse relation to where malaria cases are seen. Almost all hospitals and two thirds of health centers have malaria diagnostic capacity but only one quarter of dispensaries has it (NMCP, 2007). Yet, two thirds or malaria cases are seen in dispensaries and only one third in higher level facilities. Additionally, public health facilities are less likely to have a microscope than mission run facilities. Finally, to complicate matters, of the microscopes found in facilities only 85% were functional. As a consequence, according to National Malaria Control Programm, only between 12 – 20% of 12 million annual cases of malaria are confirmed parasitologically (NMCP, 2007).

Acridine Orange is a fluorescent dye prepared from coal and creosote oil. Stained material is observed through special fluorescent microscopes. Fluorescent dyes have

been used to stain human parasites since the 1930. It's first none experimental use with malaria dates to 1973 (Keisser, 2002). Its capacity to stain DNA and RNA has been exploited for several purposes including cancer and malaria. The malaria parasite's DNA fluoresces orange when stained with AO. The procedure, from start to result, takes between 3 – 10 minutes giving it an important advantage over Giemsa microscopy.

Modern RDT are lateral flow immune-chromatographic tests that use monoclonal or polyclonal antibodies to detect specific proteins or enzymes (i.e. antigens) produced by malaria parasites in peripheral blood. RDT detect presence of malaria parasite antigens but cannot tell the antigens from whole parasites. Some of these antigens may take up to four weeks to disappear from circulating blood and as a result can cause false positives. RDT can detect sequestered infections in internal organs, which microscopy cannot. The presence of gametocytes, which do not cause illness, but are detected by some RDT can also result in false positives. RDT are "qualitative" tests, that is, they detect the presence of antigens but do not quantify the numbers of parasites present. Microscopy on the other hand is both qualitative and quantitative. Commercially available RDT can produce a result in 15 minutes.

Besides RDTs, several other tests have been developed that do not rely on microscopy. Nucleic acid probes and immuno-fluorescence for detecting plasmodia in red blood cells; gel-diffusion, counter-immuno-electrophoresis, radio immunoassay for malaria antigens in body fluids; and hemaglutination test, indirect immune-fluorescence, enzyme immunoassay, immuno-chromatography and Western blot for anti-plasmodial antibodies in serum have all been developed.

These tests have found some limited applications in research, retrograde confirmation of malaria, investigation of cryptic malaria, transfusion blood screening and investigation of transfusion-acquired infection. Polymerase chain reaction (PCR) has been found to be highly sensitive and specific for detecting all four species of plasmodia, particularly in cases of low-level parasitemia and mixed infections. The PCR is reportedly 10-fold

more sensitive than microscopy with one study reporting a sensitivity to detect 1.35 – 0.38 parasites/µl for *P. falciparum* and 0.12 parasites µl for *P. vivax*.

Some RDT have sensitivity close to microscopy of 100 parasites /µl but there can be significant variation across types of tests and products (Gaye, 1998). Generally RDT have a sensitivity and specificity above 90%. Microscopy sensitivity and specificity under real life conditions is between 80 – 85% but it has been reported to be even lower.

For example, in some studies HRP-2 tests have detected parasitemias of 40 parasites/µl, whereas pLDH tests detect >100 – 200 parasites/µl. WHO recommends that RDT should have sensitivity ≥95% at ≥100 parasites/µl for *P. falciparum*. It should be noted that microscopy can detect 5 –10 parasites/µl when done by an experienced microscopist.

False negative results can be due to low-density parasitemia (<100 parasites/µ), interpreting the results before the time limit (15 minutes), inadequate batches of RDT or other situations. False positives can be attributed to persistent antigenia (HRP-2), detection of gametocytes when asexual forms are not present, detection of low density microscopy negative infections or presence of antigenia early in infection before parasites are detected by microscopy.

Microscopy of stained thick and thin blood smears remains the gold standard for confirmation of diagnosis of malaria. The advantage of microscopy is that, its sensitivity is high and it is possible to detect malaria parasites even at low densities. It also helps to quantify the parasite load. It is also possible to distinguish various species of malaria parasite and their different stages.

The purpose of this research is to evaluate how well RDT performs under operational conditions comparing to smear microscopy technique the gold standard.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study site

This study was conducted at reproductive and child health department in Kisarawe district hospital, Coastal region, on the Eastern part of Tanzania mainland along Indian Ocean coastal belt. The region is located between Latitudes 6° and 8° south of Equator and between Longitudes 37° and 40° East of Greenwich (National Demographic Information).

The region share borders with four regions. Northern part is Tanga region, western part is Mororgoro region, southern part is Lindi region and Eastern part is Dar-es-Salaam city. Kisarawe district is located 30 Kilometer, South west of Dar-es-Salaam region and it has surface area of 3535 sq km². It has 4 divisions, 15 wards and 74 villages. Population according to 2005 data is 101,638 and the life expectance is 49 years. (DED, 2009) Other districts of the coastal region are Mkuranga, Mafia, Rufiji, Bagamoyo, and Kibaha. The malaria prevalence of 80% is observed at the end of rain seasons because of the increase of mosquito population.

In 2004 there were 22,095 under five cases of malaria with 18 deaths making case fatality rate 0f (0.08%). By 2008 there was an increase of malaria cases up to 26,474 with 29 deaths making case fatality rate of (0.004%) (Plan International Report, 2008). The estimated infant mortality rate is 114/1,000. Estimated under five year children mortality rate is 129/1,000 and maternal mortality rate is estimated to be 450/100,000.

There is 1 public hospital, three public health centers, 13, public dispensaries, and two private dispensaries. (Source: Coast region commissioner's office, Kibaha, 2006).

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The ten most common causes of morbidity in series, includes, Malaria, Acute respiratory infections, Anemia, diarrhea, Intestinal worms, pneumonia, eye infection, skin infection, sexually transmitted infection and HIV (Kisarawe District Hospital Records, 2010).

3.2 Study population.

The study population was children with five years and below attended the Reproductive Child Health clinic at Kisarawe district hospital outpatient department with complain of fever.

3.2.1 Inclusion criteria

1. Children with five years of age and below.
2. Children with fever (body temperature $\geq 37.5\ ^{\circ}C$).

3.2.2 Exclusion criteria

1. Signs of severe illness requiring hospitalization.
2. History of anti- malarial intake in the last seven days.

3.3 Study Design

A cross- sectional study

3.4 Sample Size

A total of 285 children with five years and below were conveniently selected from the outpatient department at Kisarawe district hospital.

Sample size calculated as follows.

$$n = \frac{Z^2\, p(100-P)}{d^2}$$

Where n = sample size

Z = standard normal deviate value (set at 1.96)

That corresponded to the 95% confidence interval level.

P = proportion of malaria cases in children with five years and bellow = 25% (DED Kisarawe, 2008)

d^2= margin of error (taken to be 5% in this study).

Substitution gave a minimum sample size of 285 under five children. This was rounded up to 290 so as to accommodate the unforeseen drop outs and laboratory errors.

3.5 Sampling Technique

Convenience sampling method

By this method the sample is obtained on convenience basis for example the study units that happen to be available at the time of data collection are selected. All outpatient children with five years and below reporting at the reception with fever complain were given opportunity to be included in this study through convenience sampling. Fever was defined as those children with temperature above 37.5 Degrees Centigrade or raised body temperature within twenty four hours. The selected children were given laboratory identification number and enrolled for the test after their care taker signed the informed consent.

3.6 Recruitment and Training of Research Assistants

Four health professional staffs, a Clinical officer specializing in pediatric, a Nurse, Laboratory Technologist and Laboratory assistant were recruited for two days before the work of data collection begins. They were three males and one female professionals working at Kisarawe hospital, and were residing at Kisarawe. They were taught on blood sample collection procedure, staining and reading of results both microscopically and the RDT and interpretation of results.

3.7 Data collection and management

A structured Questionnaire was administered to obtain the demographic and the exit information on ALU treatment. After the child was seen by clinical officer, and clinical diagnosis done, they were assigned a number on their note book. In the laboratory, blood sample for RDT and thick smears for Giemsa technique were taken and labeling was done on the frosted part of the slide using a pencil. Labeling included the initials of patient name, identification number, Date, month and year in which the specimen was collected. The investigator performed all the RDT and the results were used to treat the

children. Both local laboratory technician and the third slide readers were blinded to RDT results. Stained slides were handed over to local technician with specimen tracking form where he entered his results daily. After drugs were prescribed, the patients note book was then sent back to laboratory in order to capture the treatment information from prescriber.

3.8 Laboratory procedures

3.8.1 Specimen Collection.

The children above 12 months of age totaling to 198(68 %) had their finger sterilized using cotton swab soaked in 70% ethanol alcohol then pricked with sterile lancet. For the infants 92(32 %) (Children with less than 12 months) were pricked on the heel of the foot after sterilization. One micro liter of blood was taken using a capillary tube and one thick smear was made on a clean slide simultaneously. All specimens were labeled properly using a pencil or marker pen and registered in counter book.

3.8.2 Malaria Rapid Diagnostic Test.

One micro liter of blood was placed on test window labeled `A´ on RDT cartridge and the six drops of buffer solution was dropped on well marked `B´ and results were read after exactly fifteen (15) minutes according to manufacturer instructions. Negative result for *P. falciparum* was indicated by one pink strip on the result window and positive result was indicated by two pink strips.

3.8.3 Giemsa technique.

The thin smears were fixed in absolute methanol and left to dry at room temperature. After drying, staining was done on both thick and thin smears using 10 % Giemsa working solution, diluted by buffer solution at pH 7.2. Slides were read by a qualified laboratory technician as first reader, at Kisarawe hospital daily using a good microscopy at X100 objective with oil immersion and the results were entered on structured specimen tracking form. Second reading for quality control was done by the principal investigator. The third reading was done by the qualified competent microscopist who was also blinded to previous results.

3.9 Ethical issues

The study proposal was cleared by the Research and Publication committee of Muhimbili University of health and Allied Sciences. Obligations of investigators were to adhere to all ethical issues. All information attained during the study was kept confidential. Informed consents were sought from the parents or guardians of all children included in this study and code numbers were used instead of names. Refusal to participate or withdraw from the study did not result to any kind of penalty or loss of any benefits to which non consenting participants were otherwise entitled. No risks were expected, and results were shared with hospital authority for proper clinical management of patients

3.9.1 Data analysis:

The data were entered, cleaned and analyzed in Epi info program version 3.5.1. Coefficient of variation was done in comparing results for RDT and Giemsa using reader three results. A bi-variant analysis was done in comparing results for RDT and readers one and three. Stat Calc program was used to compare the significant difference on the two readers. Chi-square test was used to test for differences between proportions. Odds ratios (OR), and 95% confidence interval (CI) were calculated and used to compare the significance of the difference.

3.10 Limitations of the study

The study was carried out during low malaria transmission season. There was time limit for the study other wise more children could have been tested.

CHAPTER FOUR

4.0 RESULTS

A total of 580 children aged 1-59 months attended the Reproductive and Child Health clinic at Kisarawe District Hospital. Of these 290 were enrolled in the study based either on history of fever in the previous night or raised body temperature on arrival. Those enrolled, had fever with body temperature >38 ^{0}C.

There were 141 (48.6%) males, and 149 (51.3%) females. The mean age was 22 months and the median was 21 months, range 1-59 months. Fever due to malaria, in Swahili ''homa ya malaria'' was the commonest symptom, 290 (100%) and this was the cause of attendance to the health facility.

Out of 290 children screened for malaria by both RDT and blood slide microscopy, 50 (17.2%) were malaria positive and were treated accordingly while the rest of the children had other illnesses which were treated by antibiotics and anti- inflammatory drugs according to the site clinician. However, 16(6.7%) were still treated symptomatically with Alu, despite having negative RDT. The remaining 290 attended due to routine vaccination as they had no complaints of fever or any other illness conditions. The flow chart for the study population is shown below.

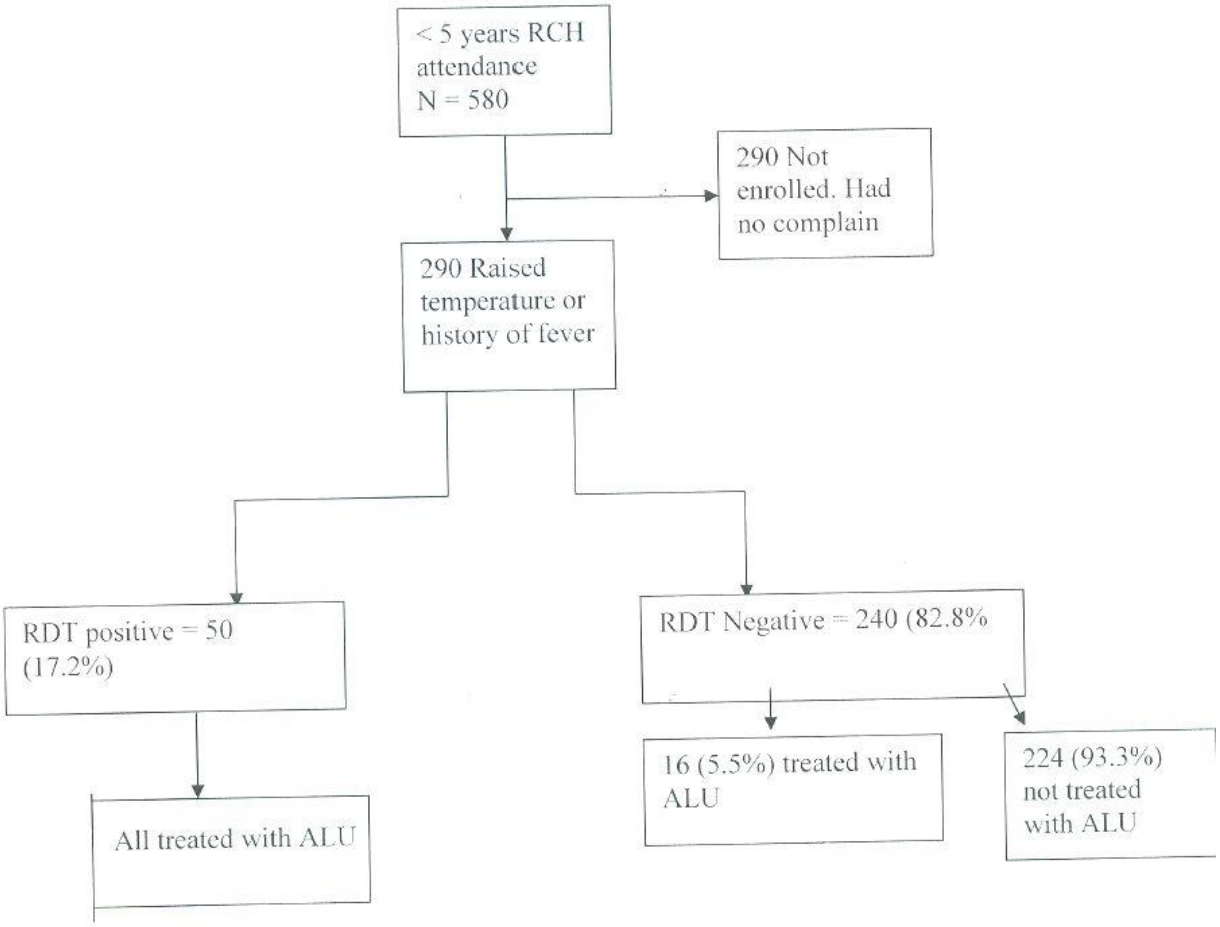


Figure 1: Flow chart of the study population.

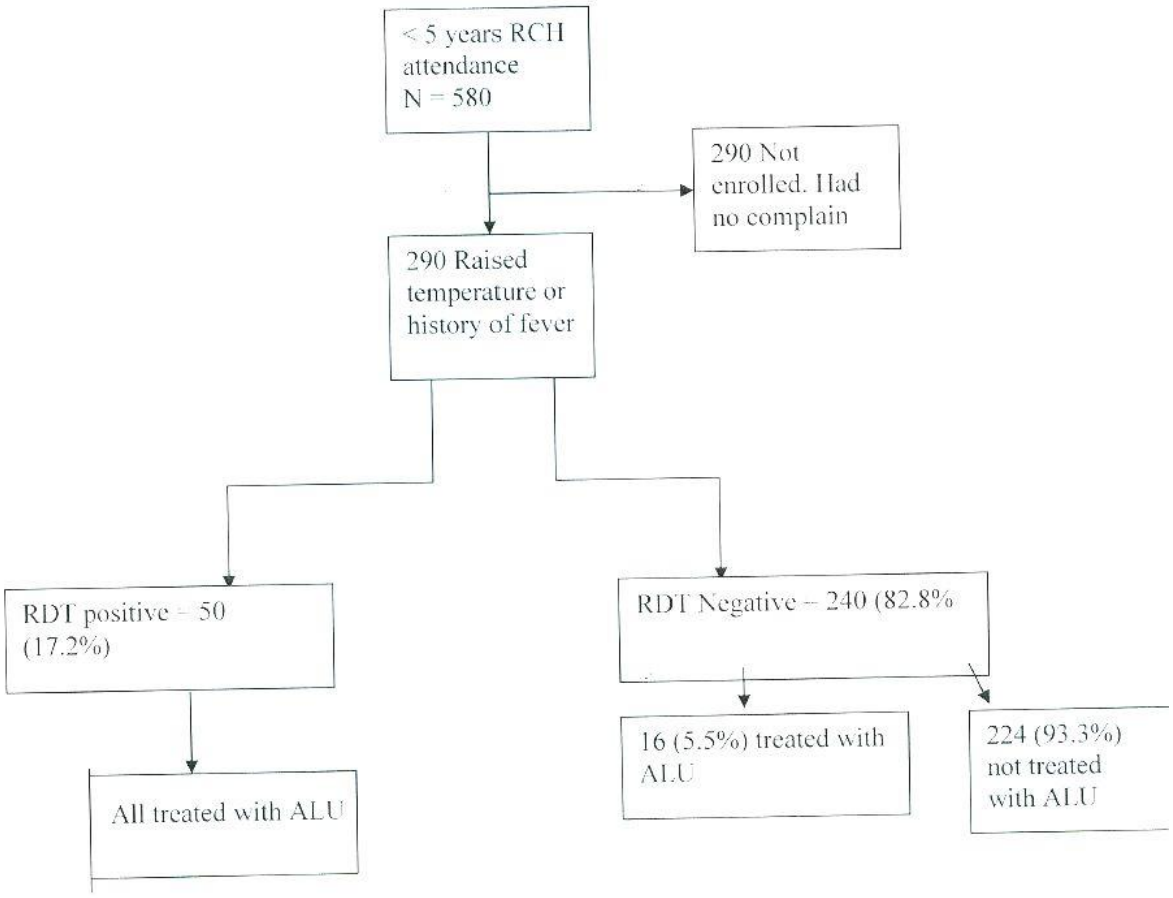


Figure 1: Flow chart of the study population.

Table 1: Age group and sex distribution of the study population

Age groups (Months)	Males n=141%		Female n=149%		Total n=290
Bellow 1 Month	7	(5.0)	2	(1.3)	9 (3.1)
1 - 11	31	(22.0)	35	(23.5)	66 (22.8)
12 – 23	38	(27.0)	36	(24.2)	74 (25.5)
24 – 35	25	(17.7)	33	(22.1)	58 (20.0)
36 – 47	18	(12.8)	23	(15.4)	41 (7.2)
48 - 59	22	(9.9)	20	(13.4)	42 (14.4)
Total	**141**		**149**		**290**

The overall mean age of the study population was 22.9 months and the median was 21 months with a range (1 – 59 months). Table one show the age groups of the study population.

Table 2: Malaria cases by sex

Sex	Malaria Positive	Malaria Negative	Total	OR	X2	P-Value
Male	26	115	141			
				1.18	0.28	0.59
Female	24	125	149			
Total	**50**	**240**	**290**			

This shows there was no statistical significant different between males and female children in getting malaria, p value = 0.59.

Table 3: Malaria symptoms, RDT positive and treated with Alu

Variable	n 290 (%)	Positive RDT (%)	Negative RDT (%)
Cough	174 (60.0)	31 (18.0)	143 (82.0)
Diarrhea	34 (11.7)	6 (17.6)	28 (82.3)
Fever	290(100)	50 (17.1)	240 (82.3)
Vomit	37 (12.7)	10 (27.0)	27 (73.0)
Abdominal pain	13 (4.5)	4 (30.8)	9 (69.2)
General body malaise	59 (20.3)	9 (15.3)	50 (84.7)
Loss of appetite	4 (1.3)	1 (25.0)	3 (25.0)

Fever (100%) and cough (60%) were the most frequent symptoms manifested by the study population at the health facility.

Clinical diagnosis

Malaria (40.7%), General body malaise (20.3%) and Pneumonia (15.2%) were the most frequent clinical diagnosis made by clinician. However, 9(13.6) pneumonia, 11(16.7%) with general body malaise, 1(1.5%) Otitis media, Flu 4(6.1%) cases received Alu. This was observed during exit interview done when patients were getting out.

Table 4: Comparison between reader 1 and reader 3 slide microscopy

		Slide reader 3		
		Positive	**Negative**	**Total**
Reader I Slides	Positive	29	17	46
	Negative	11	233	244
	Total	**40**	**250**	**290**

Kappa value:

After calculating by Kappa method for testing difference between two readers the degree of agreement between reader one smear microscopy and reader three gave kappa value of 0.6 (Normal range, 0.4-0.8) meaning there were no significant discordant.

Degree of agreement between reader 1 and 3 for parasite density

The parasite prevalence by reader three was 17% while by reader one was 13.8%. For parasite density the linear coefficient correlation was calculated. The square of the correlation coefficient was 0.86, hence the linear correlation coefficient = 0.92 which is within acceptable range.

The results of RDT and the results of the third reader had no agreement even at low parasite density (400 parasites per micro liter of blood). However reader one, the technician on site, results did not have 100% agreement with the RDT results.

Table 5: Comparison of Giemsa and RDT results

		Blood slide Reader 3		
		Positive	**Negative**	**Total**
RDT	Positive	43	3	46
	Negative	7	237	244
	Total	**50**	**240**	**290**

Sensitivity = 86%

Specificity = 99%

PVP = 93%

Accordingly the Sensitivity of RDT was 86%, Specificity 98%, and Predictive Value Negative = 97% when the gold standard is reader three.

Exit interview

A total of 66 (22.7%) children were prescribed Alu of which only 50 (17.2%) mRDT positive would have been given Alu. Based on clinical symptoms 16 (5.5%) were given Alu unnecessarily.

CHAPTER FIVE

5.0 DISCUSSION

This study was on the role of the mRDTs in the management of malaria. Malaria is the most common clinically diagnosed disease in most of African countries. In Sub-Saharan Africa malaria diagnosis is based on fever but the accuracy of clinical diagnosis is limited by the low specificity of signs and symptoms of malaria. Any child with fever is treated with anti-malaria drugs (Wang *et al*, 2006). This also is according to the WHO guidelines on the management of fever in resource poor settings (WHO, 2000)

In this study fever was the entry point based on various guidelines in which fever is used as an entry point for malaria treatment. In a holo-endemic area the accepted IMCI criteria for the treatment of malaria in children are a history of fever or documented fever (WHO, 2000). This has shown to be cost-effective in child health care but over-diagnosis becomes one amongst the problems constraining its effectiveness together with the delay of early treatment of the real cause of the fever, thus resulting in the increase of mortality and morbidity (Nicol, 2000).

RDT in the diagnosis of malaria is something we can rely on because of the sensitivity and specificity that it has in detecting the presence of malaria parasites in blood. This test detects the presence of parasite antigen (HRP-2) so the chances that diagnosis can fail is less compared to the traditional methods currently in use in which the results depend very much on the skills of the microscopist. The mRDT positivity rates varies by season and year, indicating temporal changes in accuracy of clinical diagnosis (Abeku *et al*, 2008).

On the other hand, the short coming of mRDT is that it cannot differentiate between the early and recent past infections for up to two weeks (Avila *et al*, 2002). This reflects the fact that HRP-2 can persist in the blood stream for several weeks, resulting in high false positive error rates among patients with cleared parasitaemia who seek treatment for illnesses other than malaria. Again we cannot estimate parasite density by RDT but for

screening purposes, the test is simple and time-saving. That means it is important to confirm all positive RDT cases using microscopy method.

Similar study done at Kibaha in the coastal region showed a sensitivity of 100% by ICT, and specificity of 82.0% at 95% confidence interval (Tarimo *et al.* 2000). However, another study using more specific mRDTs found a higher specificity of RDT in malaria endemic area in Tanzania; Bagamoyo in the coastal region showed a sensitivity of 90% and specificity of 99% (Premji *et al.*, 1995). The two studies were carried out during high transmission season. All these studies provided similar sensitivity with the current study however the specificity is almost the same when compared to this study which was 98 %. The difference in specificity could be due to the lower number of the study population. However, another study showed that the specificity of mRDT increases as parasite rates decreased, but sensitivity was more or less uniform among the various age groups (Abeku *et al*, 2008).

The microscopy discrepancy between reader one and three could be portraying the current challenge in the diagnosis of malaria in the Country. This has also been found in IMALIDA project done in Tanzania to assess microscopy capacity, in 12 health facilities which found a discrepancy between reader one and two of more than 40% which is more than what was observed in this study (D'Acremont, 2007).

Treatment according to RDT results

The over use of anti-malaria (Alu) has been a major problem and driving force for RTD introduction. In this study all the malarial fever positive RDT were given Alu. There was a small degree of Alu use in patients with general body malaise caused by pneumonia, flu and Otitis media. However, this over-use was not found to be statistically significant and perhaps there were reasons beyond just clinical. This has been found in other studies in which clinicians were reluctant to refrain from treating for malaria after a negative test (Zeno *et al*, 2009). Despite the fact that Clinician's adherence to a test-based strategy is a key factor in improving management of malaria and curtailing costs. Also, mRDT-based policy is usually restricted to adults and older children, while for under-

fives presumptive malaria treatment of all fevers is still recommended (Zeno *et a* 2009).

If there was no RDT, Alu treatment would have been given to all 290 children with fever, but in the presence of RDT only 66 were treated so saving of the Alu in 77%. However, even though 66 were treated actually based on RDT, only 50 should have been treated still 6.7% were given Alu unnecessarily. This indicates that training on RDT is required to ensure that only those who turned to be RDT positive, be treated with Alu, hence reduce children mortality and morbidity. Using RDT will help to avoid unnecessary treatment with anti-malarial and secondly will help in early treatment of other causes of fever if not due to malaria. This does not mean to abandon Giemsa technique but RDT can be used for screening of malaria cases in holoendemic areas and slide microscopy be used for confirmation purposes. The RDT results showed acceptable validity and even at low parasite density had good correlation with blood slide results.

6.0 CONCLUSION

Misdiagnosis of malaria is costly and results in considerable mortality because it contributes to both a delay in treatment of the correct diagnosis and to increase the rate of anti-malarial drug pressure and thus spread of resistance. The use of microscopy is challenged by misdiagnosis and clinical treatment of fever as malaria. Use of RDTs for screening all children with fever can reduce the number of unnecessary treatment of the children with anti-malaria and provide the correct treatment.

7.0 RECOMMENDATIONS

We recommend roll out and training of RDT to areas without proper microscopy infrastructure and trained personnel and if possible country wide in routine clinical setting for management of fever. Based on these findings there is need of training for both clinician and technician on the proper way of confirming malaria cases in the laboratory before treatment.

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