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Efficacy of Rapid Diagnostic Test over Blood Slide Microscopy Method used in Diagnosis of Malaria at AIC Kapsowar Mission Hospital

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Abstract

World Health Organization (WHO) estimates that 3.2 billion people are at risk of malaria worldwide with Sub-Saharan Africa having 88 percent of malaria cases and 90 percent of malaria deaths. In Kenya, malaria remains a major cause of morbidity and mortality with more than 70 percent of the population at risk of the disease. Malaria is caused by protozoa parasites of the genus Plasmodium. It is diagnosed microscopically by staining thick and thin blood films. It requires a lot of experience and expertise to diagnose malaria when at low levels. In addition, it is difficult to microscopically diagnose malaria in areas with no electricity. With the introduction of rapid diagnostic tests, malaria diagnosis in rural and remote areas is possible. It is thus important to test efficacy of rapid diagnosis tests in Kapsowar Mission Hospital with the aim of rolling out the usage of the kits. The objectives of the study were to test the efficacy of rapid diagnostic test kits and determine the prevalence of malaria in patients attending AIC Kapsowar Mission Hospital. Informed consent was sought from patients to be included in the study. The patients' history was taken and a blood sample drawn to test for malaria. Two tests were performed on each sample which included a thick smear stained with giemsa and examined via microscope to determine presence of malarial parasites and a parallel test using the rapid diagnostic test kits. Data collected was entered and analyzed using SPSS V.16.0 for windows. Descriptive statistics were used to summarize the data and subjected to t-test and statistical significance level determined at $p < 0.05$. The sensitivity of rapid diagnostic test kit was found to be 81% while the specificity was 87%. The prevalence of malaria was found to be 38% with more children and pregnant women having the highest confirmed cases of malaria. The study recommended that rapid diagnostic test kits be availed in remote areas for more efficient and rapid diagnosis of malaria.

Keywords: ***Malaria, giemsa stain, rapid diagnostic test, sensitivity, specificity***

Introduction

Malaria is one of the successful parasites causing one of the most debilitating diseases ever known to mankind. After thousands of years, it remains the world's most pervasive infection affecting several countries and about 30 million people worldwide. It is a tropical disease and countries in Sub-Saharan Africa account for nearly 90% of all malaria cases (WHO, 2013). Malaria causes up to 10 million deaths worldwide and in Africa it accounts for 25% of all deaths of children under the age of 5 (Ofori et al., 2009). Malaria is caused by a protozoan parasite of the genus plasmodium which is usually transmitted by Anopheles mosquito bite. The

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disease is characterized by fever, shivering joint pain, and headache and vomiting in severe cases. The patient may develop jaundice, kidney failure and can lapse into coma in severe cases.

Malaria is common among young children and pregnant women (Cheesbrough,2006). Microscopy remains the gold standard method for the detection of malaria parasites. During the Malaria eradication (ME) era, microscopy was the mainstay of malaria development. Despite the organizational and logistic challenges, residents of originally malaria infested areas successfully eliminated the disease. The detection threshold in Giemsa stained blood film has been estimated to be 4-20 parasites/ inch. Poor microscopy has long been recognized in failure to detect the parasite and is a function of multiple factors such as poor training and skills maintenance, slide preparation, workload, condition of the microscope and quality of essential lab supplies. Microscopy has poor specificity and sensitivity and poor blood film preparation generates artifacts such as bacteria, dirt and cell debris. Normal blood components such as platelets can hamper diagnosis and therefore improved training and high quality of smear preparation is required to reduce false positive results.

WHO recommends that Rapid diagnostic test (RDT) should be at least as accurate as results derived from microscopy performed by an average technician under routine field conditions (WHO, 2013). Specificity should be above 95% compared to microscopy and should be detected reliably with a sensitivity of 100%. RDT's were introduced in early 1990's and have the following advantages; minimal operator training, ease of platform 2 with minimal steps, reproducibility of results, rapid availability of results (<20 minutes) and low cost (WHO, 2011). Malaria RDT needs a high sensitivity of Plasmodium falciparum, but specification is required to avert inflated estimate of the burden of malaria, misperceptions of inadequate therapeutic responses when fever is due to other illnesses and unnecessary drug pressure. Malaria RDT's employ immunochromatographic technology similar to rapid pregnancy tests. In these assays the sample migrates in liquid across the surface of a nitrocellular membrane by capillary action. For a given targeted parasite antigen two sets of monoclonal or polyclonal antibodies are used; RDT's are commercially available (Lokom, 1996).

The majority of malaria cases occur in countries where cost-effectiveness of the test, ease of diagnostic test performance and training of personnel are major considerations. Most new technologies for malaria diagnosis incorporate immunochromatographic capture procedures, with conjugated monoclonal antibodies providing the indicator for infection. Preferred targeted antigens are those which are abundant in all asexual and sexual stages of the parasite. Current interest is focused on the detection of histidine-rich protein 2 (HRP-2) from Plasmodium falciparum and parasite-specific lactate dehydrogenase (pLDH) or Plasmodium aldolase from the parasite glycolytic pathway found in all species. HRP-2 is a water-soluble protein antigen produced by asexual stages and young gametocytes of P. falciparum (Kyabayinze, Tibenderana, Odong, Rwakimari & Counihan, 2008; WHO, 2011). It is expressed on the RBC membrane surface and

because of its abundance in P. falciparum and was the first to be used in RDT. However, pLDH, an enzyme found in the glycolytic pathway of the malaria parasite, is produced by sexual and asexual stages of the parasite (McCutchan, Piper & Makler, 2008). Different isomers of pLDH for each of the four Plasmodium spp. infecting humans exist, and their detection constitutes a second approach to RDT development. Several other enzymes of the malaria parasite glycolytic pathway, notably aldolase (WHO, 2011), have been suggested as target antigens for RDT for species other than P. falciparum. RDT is commercially available in two forms; either dipstick format or card test kits for the detection of malaria antigens.

Statement of the Problem

In Kenya malaria remains a major cause of morbidity and mortality accounting for 30% outpatient and 19% admission cases to health facilities (MOH, 2006). Microscopy has for a long time been and used in diagnosis of malaria. However, it has been criticized for ineffectiveness due to inadequate training of personnel, poor slide preparation, condition of microscope and quality of essential laboratory supplies, cost of the test and the mandatory electricity requirement which is not attainable in rural health centers. It is therefore necessary to explore an alternative sensitive and specific method that is cost effective and providing accurate diagnosis even in remote areas without electricity. Malaria has also been shown to vary in severity and prevalence with regard to host factors such as age, sex and pregnancy status. However, the influence of these factors in relation to malaria has not been established in the region.

Objectives

1. To determine the specificity and sensitivity of Rapid diagnostic Test (RDT) over Microscopy.

2. To determine the point prevalence of malaria among the patients attending Kapsowar Mission Hospital.

3. To determine the prevalence of malaria infection in relation to age, sex and pregnancy status.

Justification

The research findings will help policy makers, donors and health professionals to utilize results of this study for designing appropriate interventions.

Materials and Methods

Site of Study

The research was conducted in A.I.C Kapsowar Mission Hospital, a faith based hospital situated in North Rift region, Keiyo-Marakwet County and serves the nomadic communities of Marakwet, Pokot and Turkana.

Study Population and Research Design

The study was a cross-sectional descriptive study involving all febrile patients sent to the laboratory for malaria test.

Sample Size Determination

The fishers' exact formula (Mugenda, 2003), was used to obtain the sample size as follows;

$$n = \frac{\mathrm{t2} \times \mathrm{P}(1 - \mathrm{P})}{\mathrm{m2}}$$

Where n = required sample size

t = confidence level at 95 %(standard value of 1.96)

P = estimated prevalence of malaria of 10% (A.I.C Kapsowar Mission Hospital records)

m = margin of error at 5 % (0.05) Substituting in the above formula, n = 231

Sampling Technique and Data Collection

Eligible patients were sampled consecutively as they came to the hospital for treatment. Questionnaires were used to establish demographic characteristics such as age, sex and pregnancy status. Clinical history was taken to capture any signs of malaria, age and sex. Informed consent was obtained from the patients and/or their parents/guardians.

Blood samples were collected aseptically from a finger prick done using a sterile needle. This blood was collected using EDTA capillary tube ready to be transferred to the various test kits for examination.

Laboratory Methods

Microscopy. A blood smear was made by dropping 50µl of blood on a microscope slide using the capillary tube and spread to make a thick film then air dried for 10 minutes after which the thick smear was stained using Giemsa and examined under a light microscope under x100 magnification to look for malaria trophozoites. All results were recorded for comparison with the RDT.

Rapid Diagnostic Test (RDT). From the capillary tube, 40µl was dropped on the sample well of the RDT kit and left to run for 15 seconds after which, two drops of chase buffer were added and results read within 15minutes. Formation of two lines on the result and control area respectively is considered positive whereas one line on the control area is negative. Any test kit that shows one line on the result area but none on the control area or has no line at all is considered as negative.

Data Analysis

Data collected was entered and analyzed using SPSS V.16.0 for windows. Descriptive statistics (frequencies, means and standard deviation) was used to summarize the data which was then subjected to correlation and t-test to determine whether there were significant relationships or differences between two groups. The statistical significance was determined at $p < 0.05$

Results

Prevalence of Malaria in A.I.C Kapsowar Mission Hospital

The findings showed that out of 231 (100%) patients that were enrolled in the study,

88 (38%) were positive for malaria giving a prevalence of 38%. Most of the cases were reported during warm humid weather. The true positives were 69 (78.4%) and the true negatives were 127 (88.9%). The false positivity and negativity was 19 (21.6%) and 16 (11.2%) respectively as shown below.

Table 1 *Specificity / Sensitivity of the Rapid Diagnostic Test*

		Microscopy				
		Positive	**Negative**	**Total**	**Sensitivity %**	**Specificity %**
Rapid Diagnostic Test	Positive	69(78.4)	16(11.2)	85	81	
	Negative	19(21.6)	127(88.9)	146		87
Total		**88(38.1)**	**143(61.9)**			

Using the formula [{True negative / (True negative + False positive)} x 100] the specificity of rapid diagnostic kit was found to be 87%

Positivity and Negativity of Microscopy versus Rapid Diagnostic Test for Malaria

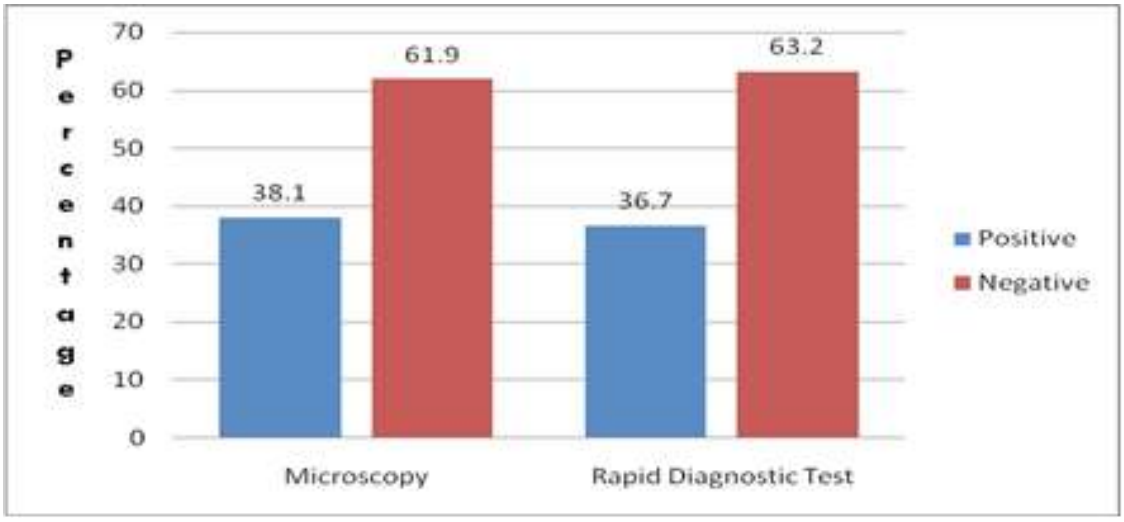

Figure 1 *Comparison of the positivity and negativity of microscopy and rapid diagnostic test for malaria*

Results showed no significant differences between sensitivity and specificity of microscopy and RDT. Both methods are effective in detection of malaria positivity and either of the two can be used wherever possible.

Table 2 *Host factors associated with Malaria*

Variable	Positive	Negative	Total	p value
	No (%)	No (%)	No (%)	
Age				
0-19	47	68	115	
10-19	24 (42.1)	33 (57.9)	57 (24.6)	0.001
30+	17 (28.8)	42 (71.2)	59 (25.5)	
Total	88 (38.1)	143 (61.9)	231 (100)	
Sex				
Male	31 (33.6)	73 (66.4)	110 (47.6)	0.025
Female	51 (42.1)	70 (57.9)	121 (42.4)	
Total	88 (38.1)	143 (61.9)	231 (100)	
Pregnancy				
YES	29 (59.2)	20 (40.8)	49 (40.5)	0.075
NO	22 (30.6)	50 (69.4)	72 (59.5)	
Total	**51 (42.1)**	**70 (57.9)**	**121 (100)**	

Discussion

Prevalence of malaria in Elgeiyo Marakwet region served by Kapsowar Mission Hospital was 38%. This was in line with the WHO estimates for Kenya, which indicate that 15 million out of 40 million Kenyans have malaria (approx 37.5%). Other tropical countries report similar range in prevalence (Arevalo-Herrera, Quinones, Guerra, Cespedes & Giron, 2012). Malaria cases in Kenya show a seasonality trend with high numbers usually recorded during the cool to warm and humid season and fewer or no cases during the hot and dry season. Malaria cases usually range from mild to severe cases depending on the level of parasitemia.

The sensitivity and specificity RDT in the study was 81% and 87% respectively for Plasmodium falciparum. Although specific kits were not used for other species of Plasmodium, previous findings have confirmed that new immunochromatographic antigen tests are capable of detecting 100 parasites/l (0.002% parasitemia) and of giving rapid results within 15 to 20 minutes. Although RDT kits have not been used

widely in Kenya, the commercially available kits which have been hailed for ease of performance of the procedures and the non-requirement for elaborate training or equipment to perform or interpret the results (WHO, 2013). Some studies have suggested that, to be a useful diagnostic, RDTs must achieve greater than 95% sensitivity (Jiang, 2010), but noted that in countries where RDTs have been applied, this level of sensitivity and specificity has been achieved for P. falciparum, but not for non-P. falciparum. For instance, in the evaluation of an HRP-2 prototype assay in Thailand and Peru, P. falciparum sensitivity was found to be 100% for parasite density < 500/mcL and 83% for < 500/ mcL.66. RDT sensitivity declines at parasite densities < 500/mcL blood for P. falciparum and < 5,000/mcL blood for P. vivax. False positive RDT results have been found to occur in a few tests and have been attributed to cross-reactivity with rheumatoid factor in the blood but replacement of IgG with IgM in recent products in the course of infection is noted to reduce this problem (Ngasala et al, 2008).

An evaluation of factors affecting prevalence of malaria showed that age, sex and pregnancy had significant influence on malaria positivity. Malaria is a major cause of casualties in children under five years due to their less developed level of immunity which leaves them more vulnerable to disease. Results also showed that more women 51 (58%) than men 31(42%) were positive for malaria using RDT which was a significant difference recorded (Chi square, $p<0.05$). In the pastoral communities of Kenya, it is evident that whereas boys and men tend to livestock in grazing pastures, women undertake several indoor and outdoor activities such fetching water, firewood and tending to farm crops in the forested areas where exposure to mosquito bites is likely to be higher. Theses cultural practices are common in most nomadic pastoral communities in Africa. Pregnancy was also found to impact positively on malaria positivity $p<0.05$). Some studies have suggested that the use of microscopy in diagnosis of malarial infection in pregnant women can pose some challenges due to placental sequestration of parasites thus reducing the sensitivity of microscopy (Ofori et al; 2009) but the detection of peripheral blood HRP-2 genes is possible using RDT. The ability to detect placental infection by antigen detection when microscopy does not identify parasitaemia may have a significant impact on maternal and fetal health care (Murray, Gasser, Magill, & Miller, 2008). Generally, widespread use of malaria RDT may lead to increased opportunity for cost savings if it is introduced in health facilities especially in rural communities where microscopic examination for malaria diagnosis is not readily available.

Conclusions

Prevalence of malaria in AIC Kapsowar Mission Hospital was 38% and the sensitivity of RDT over microscopy was is 81% while the specificity is 87%. Age, sex and pregnancy status had a significant effect on malaria positivity.

Recommendation

The Malaria Rapid Diagnostic Test is a verifiable tool for parasite based diagnosis

of malaria and is highly recommended for use especially in the rural communities with inadequate facilities and technical staff.

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