ORIGINAL PAPER

Effectiveness of Rapid Diagnostic Test for Malaria Diagnosis in Children under 15 Years of Age of Nchelenge District in the Luapula Province

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ABSTRACT

***Objective**:* To compare the Effectiveness of Rapid Diagnostic Test (RDT) to Microscopic examination in detecting malaria parasites.

***Design**:* A cross sectional method was used in this study. The researcher collected information from sampled individuals using quantitative and qualitative methods. Three (3) Health centres in Nchelenge District were randomly selected. Thereafter, a systematic sampling procedure picking every 2nd child was used to select children (under 15 years of age), attending the diagnostic centres.

***Main Outcomes**:* The figures for specificity, sensitivity, and predictive values were calculated in percentage using microscopy as the gold standard.

***Results**:* The findings of this study demonstrated that the RDTs for detection of *malaria P. falciparum* was highly sensitive (96.1%) but less specific (53.8%) for the diagnosis of malaria plasmodium falciparum, with a positive predictive value (PPV) of 80.2% and a negative predictive value (NPV) of 87.6%.

The sensitivity of the HRP-II RDTs correlated directly with parasite density and it was 100% at parasitemias of above 440/µl; however, the sensitivity dropped from overall 98 to 50% at parasitemias of less than 440/µl.

***Conclusion**:* This study demonstrated that 96.1% of RDTs can diagnose malaria *P.f.* in an individual whether symptomatic or not. The Ministry of Health through the NMCC is intending to bring down the prevalence of malaria substantially by the year 2011; this target can be achieved if RDTs that have 96.1 % sensitivity are used as a major diagnostic tool in all the districts of Zambia.

INTRODUCTION

Malaria continues to be one of the main public health problems in the world, especially in a majority of African countries. WHO[12] estimates 1,272,000 deaths to have occurred globally and Africa was leading with; 1,136,000 (89.3%), South-East Asia; 65,000 (5.1%), Americas; 1,000 (less than 1%), Western Pacific; 11,000 (less than 1%), Eastern Mediterranean; 59,000 (4.6%), Europe; 0 (0%). To overcome malaria challenges, there is a need for concerted efforts in the management of malaria cases using accessible and rapid diagnostic tools by health services, private sector, local and international communities.

Recently, rapid antigen detection methods have been developed for situations in which reliable microscopy may not be available. These tests are based on the detection of antigen(s) released from parasitized red blood cells [6]. In the case of *Plasmodium falciparum*, these new methods are

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based on detection of *P. falciparum* histidine-rich protein 2 (HRP-2) (*Para*Sight F, Australia or *Plasmodium*-specific lactate dehydrogenase (pLDH)). Species-specific pLDH isoforms have been used to develop a test for *Plasmodium vivax* (OptiMAL) [6].

Paracheck is one of the rapid tests that detect the presence of Plasmodium falciparum (*P.f*) from the finger prick blood sample; it utilises the principle of immunochromatography in blood. It is an indirect method of detecting P.f as it detects the HRP-2 antigen secreted by the trophozoite and gametocyte the moment that they invade the red blood cells. As the test sample flows through the membrane assembly of the dipstick after placing into the clearing buffer tube, the colored anti P.f HRP-2 antiseracolloidal gold conjugate (monoclonal) complexes the P.f HRP-2 in the lysed sample. This complex moves further on the membrane to the test region where it is immobilised by the anti P.f.HRP-2 (monoclonal) antisera coated on the membrane leading to formation of a pink colored band which confirms a positive test result. Absence of this colored band in the test region indicates a negative test result. The unreacted conjugate and unbound complex if any, move further on the membrane and are subsequently immobilised by anti rabbit anti bodies coated on the membrane in the control region, forming a pink band. This control band serves to validate the test performance [1].

The appropriateness of microscopy versus antigen-detecting rapid diagnostic tests depends on a number of factors including parasite prevalence, availability of skilled personnel and resources, and the capacity for maintaining quality assurance of microscopy and RDT, and the need for quantitative assessment of parasite density [3]. RDTs that are well kept in good condition can achieve sensitivity similar to that commonly achieved by microscopy. Sensitivity can vary between products but the recommended sensitivity is 95% at 100 parasites /µl for P. falciparum [13].

Rapid diagnostic tests, in simple kit form, can provide results based on fingerprick or venous blood within minutes. They can be used by village health workers after as little as an hour of training (Makler and Palmer, 1998). The RDT result is obtained within 10 minutes using a very simple technique. Its greatest disadvantage is that it remains positive for seven to 14 days after treatment. There is, thus, no point in re-doing the test during that period [14].

METHODS

A cross sectional method was used including quantitative techniques of data analysis. The sample was drawn from children under 15 years of age attending the laboratory facilities in Nchelenge District of the Luapula Province. Three (3) health centres in the district were randomly selected. Thereafter, a systematic sampling procedure picking every 2nd child was used to select children (under 15 years of age), attending the diagnostic centres and each Health centre had equal numbers of sample and the hospital OPD sample size doubled other centre's number. The sample size required was estimated to be 437 assuming 95% a confidence interval, 90% response rate, 80% sensitivity and 90% specificity. Out of the total sample of 427, 48 were removed for they did not meet the laboratory standards leaving a total sample of 389 respondents.

Peripheral blood smear examination for malarial parasites was collected at the same time for both blood slide and RDT for each child under 15 years of age who voluntarily participated in the study. Each respondent was asked about signs and symptoms of malaria and medication taken during the prior 3 weeks. Using the edge of slide and another with the corner of slide made thick and thin blood smears. Slides were stained with Giemsa stain 10% and examined under light microscope. Thick smears were examined for presence of parasite, while thin smears were evaluated for species of parasite. At least 100 high power fields were scanned in each slide. Two (2) experienced laboratory technicians (who have been consistantly dealing with the blood slides for at least the past 4 months prior to this study) examined each blood side for all the samples independently and were blinded of the RDT results, and a 3rd was used to ascertain results that were found conflicting. Results of thick and thin smears were recorded on the questionnaire whether they were positive or negative for malaria for each child and the type of plasmodium was also recorded.

For the same finger prick, Paracheck *Pf* strips testing the presence of HRP-2 antigen were used in the

study. The Paracheck strips were brought to room temperature and finger prick blood delivered to its designated area of the dipstick. Two-three drops of clearing buffer was dropped on to the sample padjust below the arrows on the dipstick as manufacturer instructions. After 15 minutes, the readings were noted. The test was considered valid if only one control pink color line was visible on the dipstick and as positive for falciparum, if the second HRP-II line of distinct color bands appeared.

Ethical clearance was requested and granted from the UNZA Research Ethics Committee. Permission from the Authorities of District and Health Centres concerned was obtained. Data collection was done within the months of March and April 2008. The data was analysed using EP Info V6, confidence interval was done at 95% for sensitivity and specificity. Sensitivity and specificity and negative and positive predictive value were done using the following standardised table:

Reference Test Results (Microscopy)
New Test Results (RDT)

	Positive	Negative
Positive	***True Positive (TP)***	***False Positive (FP)***
Negative	***False Negative (FN)***	***True Negative (TN)***

Table 1: Sensitivity, specificity, positive and negative predictive values were calculated as follow;

- ***i. Sensitivity: TP/ TP+ FN,***
- ***ii. Specificity: TN/ FP+ TN,***
- ***iii. Negative Predictive Value: TN/ TN + FN, and,***
- ***iv. Positive Predictive value: TP/ TP+ FP.***

FINDINGS

This study tested the accuracy of paracheck *Pf* brand of HRP-II RDT for *P falciparum* compared to microscopy, with samples from symptomatic and non symptomatic suspected malaria clients. This study was conducted in a hospital out patient department and two Health Centers of Luapula Province, Nchelenge District (Zambia). Among children included in the study, 190 (48.8%) were recorded from St Paul's Out Patient Department, 101 (26%) were recorded from Kashikishi Rural Health Centre and 98 (25.2%) were recorded from Nchelenge Urban Health Centre. The children ranged in age from 8 months to 14 years with an average age of 6.3 years.

Among the samples, positive blood slide for malaria (use of microscopy) were found in 257 (66 %) children with the remaining slides 132 (34 %) found negative. All the samples revealed *P falciparum*. The HRP II antigen detection Paracheck Pf for the rapid diagnosis of falciparum malaria was used in this study and out of the total respondents, the majority 308 (79.2%) became positive and 81 (20.8%) were RDTs negative, giving a higher percentage of positive results than the microscopy results (table 2) but closer to respondents who presented with signs and symptoms of malaria 342 (88 %). Out of these, the majority 316 (79 %) had fever, and out of those who had fever, 265 (86 %) were RDT Positive (Table 1), making fever the core pre-determining sign of malaria after ruling out other illnesses that may cause fever [10].

TABLE 1: Respondents' RDT results and their history of Fever

History of Fever within the previous 2 weeks	RDT Positive		RDT Negative		Total	
	Freq	%	Freq	%	freq	%
Yes	265	86	51	63	316	79
No	43	14	30	37	73	21
TOTAL	308	79	81	21	389	100

TABLE 2: Comparing RDTs to microscopy results

RDTs	*Microscopy results* Positive		Negative		*TOTAL*	
	Freq	%	Freq	%	Freq	%
Positive	247	96.1	61	46.2	308	79.2
Negative	10	3.9	71	53.8	81	20.8
TOTAL	257	66	132	34	389	100

The sensitivity of RDTs in this study was 96.1% with 95% CI of 93.7 – 98.5, and the specificity was 53.8% with 95% CI of 45.3 – 62.3, Positive Predictive Value was 80.2% and Negative Predictive Value was 87.6 % (2). These results show that the sensitivity is comparable to other studies' findings that range between 73.7% and 100% (100% [7]; 98% [9]; 91.2% [2]; 80.25% [4]; and 73.7% [11]). Results from this study show that the use of Paracheck-*Pf* is sensitive and as good as microscopy in detecting falciparum malaria cases. This is paramount, as true malaria cases would be captured and treated on time in this vulnerable population of children 0-15 years of age.

The majority of the blood slide positive respondents 169 (65.8%) had parasite density ranging from 40-200/µl and those who had parasite density of 40 parasites/µl were 100 (22.9%). This could explain that a good number of false positives may have had less than 50/µl parasite density. An independent expert microscopist was sought and requested to increase the reading fields to more than 500 and 10 negative blood slides that were matching with the RDTs positive were randomly selected and re-examined, only 1 (10%) came up positive with a parasite density of 16/µl and this could also explain that more blood slides could have had parasite density below the parasite threshold level. Moreover, in *P. falciparum*, the parasite density may also be difficult to find since they disappear from the peripheral blood after 24-26 hours of asexual development as a result of adherence to infected erythrocytes to the endothelium of venule and capillaries in the vital organs [8]. Thus, if peripheral smear is examined after this stage, which could have occurred in this study's samples, it may not detect parasite but the HRP-II testing the malaria antigen is not likely to miss the diagnosis. Another reason for false positivity with RDTs can be that of previous malaria episode in the last 3 to 4 weeks but this may not account to the majority of the cases in this study because the investigator took care of it by excluding all those that had a history of malaria whether treated or not.

The performance of RDTs was influenced by the level of parasitemia in peripheral blood, The sensitivity of the Paracheck p.f HRP-II RDTs was 100% at parasitemias of above 440/µl; however, the sensitivity dropped from 98 to 50% at parasitemias of less than 440 to 40/µl. These study findings are consistent with earlier findings describing that the sensitivity decreases in correlation with the decrease in parasitaemia [4,2,11]. This can potentially be dangerous, as to miss the diagnosis of malaria in an ambulant patient may mean that complications develop because appropriate treatment was not instituted in time.

CONCLUSION

The Rapid Diagnostic Tests (RDTs) are rapid and simple to perform and to interpret by anyone including Community Health Workers. This shows how desirable these public health tools are to alleviate the diagnostic challenges faced by many people who can not access microscopic services for malaria diagnosis in Zambia. This study results add to the evidence that these non-microscopical rapid tests for the detection of plasmodial antigens may develop into important diagnostic tools and can prove to be a valuable adjunct to clinical assessment of the patient and blood film microscopy under certain circumstances.

The study findings demonstrated that the sensitivity of the RDT was high (96.1% with 95% CI 93.7 – 98.5) and it is comparable to other studies that were done previously. These findings confirm that the RDTs are very effective as diagnostic tools and can be a substitute where microscopy facilities are unavailable. Apart from its sensitivity, there are so many advantages, RDTs are easy to use, they don't require expertise, require less efforts, and you can have results within 10 – 15 minutes (unlike microscopy that takes more than 45 minutes to come up results), and they can find malaria parasite antibody as soon as the parasites invade the RBCs within the fist 24 hours even when the parasites density is very low [7].

This study's specificity results (53.8% with 95% CI 45.3 – 62.3) is relatively less compared to other studies that were population based, however, it raises particular evidence that study done in clinical settings are likely to have less specificity of the RDTs HRP-II for Malaria infection and this increases the chances of spending more on unnecessary medication. The parasite density shows that the majority of respondents 169 (65.8%) had parasite density ranging from 40-200/µl. Parasitaemia influences the sensitivity such that

higher the parasite density higher are the chances of RDTs becoming positive.

The study findings also revealed that there were more false positive {61 (46.2%) of the total RDT positive 308 (79.2%)}which explains the outcome of a relatively low specificity. This was attributed to low parasite density that might have resulted from limitations of microscopy, especially the parasite sequestration limiting the number of circulating parasites in peripheral blood but on the other hand the HRP-II are less likely to be missed by the RDTs.

RECOMMENDATIONS

This study revealed a number of issues that need to be worked on and the following recommendations were made:

- Clients are likely self-medicate themselves and may fear to tell the truth to the health providers. Therefore, more efforts to improve on health education about the RDTs should be intensified.
- The Zambia Ministry of Health plans to reduce the malaria burden by 70 to 80 percent through efforts to scale up malaria control interventions. This public health task can not be realised without malaria diagnostic tools such as RDTs. Therefore, the need to scale up the provision of these tools in all public health facilities.
- More health care providers need to be reoriented and trained on the use of RDTs, for acceptability and compliance in the implementation of these important public health tools. The Paracheck RDT has demonstrated a high sensitivity (96.1%) and so may improve areas that goes with use of this tool; RDTs are unable to miss the malaria parasite antigen compared to microscopy that can miss parasites below the 50/µl threshold level, and the RDT are capable of detecting the parasite antigen within the early hours of infections, within 24 hours time, as soon as the parasites enter the Red Blood Cell where as microscopy may fail to detect parasites within the first 32 hours after infection. The Paracheck P.F RDT has shown strong performance as they can test the malaria antibody even when the parasite threshold is below microscopic detection level.
- Currently in all the Districts, the Government of Zambia has committed itself to use RDTs as diagnostic tool for improving diagnostic confirmation of malaria cases and case management. The study has demonstrated that 96.1% of RDTs can diagnose malaria *P.falciparum* in an individual whether symptomatic or not. The Ministry of Health through the NMCC is intending to bring down the prevalence of malaria substantially by the year 2011. This target can be achieved if RDTs that have 96.1 % sensitivity are used as a major diagnostic tool in all the Districts of Zambia.

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