

Original Article

The use of Rapid Diagnostic Test (ParacheckPf) in an area of low malaria transmission, Khartoum, Sudan

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Abstract

A cross-sectional hospital-based descriptive study was carried out in Bashair teaching hospital in Khartoum, Sudan, in order to assess the use of ParacheckPf test for detection of P. falciparum histidine-rich-protein2 (HRP2) in malaria case management.

A total of 164 patients (mean age 19 (\pm 16.5)) presented with fever, or history of fever (within the previous 3 days) were included in the study. Patients/caregivers were interviewed and all patients were physically examined; for each patient blood smears were examined microscopically and a ParacheckPf test was performed. ParacheckPf test was positive in 6 (3.7%) of the patients, of this asexual stage of P. falciparum was detected in 5 (3%). All laboratory-confirmed (microscopy and/or ParacheckPf test positive) malaria patients were males and \geq 5 years of age. History of fever was stated by all microscopically confirmed 5 (100%) and by 5 (83.3%) with ParacheckPf positive reaction. All laboratory-confirmed malaria patients were treated according to national protocol for malaria treatment. Furthermore, Artesunate plus Sulfadoxine-pyrimethamine (AS+S/P) was prescribed for 12% (19) of 158 patients) with laboratory negative result. Data showed no differences between negatives and confirmed malaria (microscopy/ RDT), for means (\pm SD) of duration of illness, axillary temperature and previous attack.

This study showed reasonable concordance between microscopy and the ParacheckPf test. The study revealed that ParacheckPf is relatively accurate in diagnosing falciparum malaria and can be used in hospitals where microscopy is inaccurate.

Keywords: ParacheckPf, Malaria, Khartoum, Sudan

Introduction

Early diagnosis and treatment are vital keys to address morbidity and mortality due to malaria⁽¹⁻³⁾. Microscopic detection of malaria parasites in thick blood smears remains the most appropriate diagnostic method in endemic countries. However, microscopy does need well qualified and supervised laboratory workers, maintenance of the microscope and regular supply with consumables, conditions which are difficult to sustain in many sub-Saharan countries⁽⁴⁾ this was proved to be the situation in different parts of Sudan. Health authority for extended period was trying to improve microscopic

diagnosis of malaria through a quality assurance system but without any apparent change. Therefore, they started to look for ways to solve this problem through reliable and practical alternative tool. The only feasible solution at present is the use of malaria Rapid Diagnostic Tests (RDTs). Malaria RDTs assist in the diagnosis of malaria by providing evidence of the presence of malaria parasites in human blood; they are easy to perform, do not require extensive training or equipment^(5,6). Malaria antigens currently targeted by RDT are histidine-rich-protein (HRP2)⁽⁷⁾, parasite lactate dehydrogenase (pLDH)⁽⁸⁾ and Aldolase⁽⁹⁾.

The recent change in treatment policies to more expensive multi-drug regimes are increasing the importance of obtaining an accurate diagnosis based on demonstration of parasitaemia prior to treatment ⁽¹⁰⁾. In Sudan, *Plasmodium falciparum* accounts for about 98% of the malarial infections (Malaria prevalence and coverage indicators survey, Sudan, 2005). As recommended by National Malaria control programme (NMCP) RDTs are to be used in situations where microscopy is unavailable, impractical or inaccurate. Therefore, it is crucial at present to assess the use of this method for the diagnosis of malaria. Accordingly, this study was carried out in order to assess the use of a ParacheckPf for detecting *P. falciparum* HRP2 for confirmation of malaria in patients attending a hospital

Objective

To assess the appropriateness of use of ParacheckPf in the diagnosis of falciparum malaria in an urban area of low transmission in Sudan

Subjects and methods

A cross-sectional hospital-based descriptive study was carried out in Bashair teaching hospital in Khartoum, Sudan during the period from 6th July to 4th August 2006 (period of low malaria transmission in Khartoum). All malaria suspected patients during the study period were included.

Data collection

The study started by meeting with some of the hospital staff, explaining to them the aim of the study. Patients presented with fever (i.e. an axillary temperature of ≥ 37.5 °C), or history of fever (within the previous 3 days) after excluding obvious reasons of fever were selected. After obtaining informed consent of the patient or caregiver, a pre-tested questionnaire was used to collect demographic and clinical data. Patients were interviewed and physically examined by the clinicians.

Further, from each patient thick and thin blood smears were prepared and stained with 10% Giemsa stain. From the same finger prick blood was collected and ParacheckPf device (Orchid Biomedical System, Goa, India; Lot No. 21114; exp. Date August 2006) was used for testing whole blood for *Plasmodium falciparum* HRP2. The test was performed strictly according to manufacturer's instructions. Devices were stored according to recommended temperature (between 4 °C and 30 °C) prior and during the study period ⁽¹⁰⁾.

Microscopic examination of 100 thick-blood-smear fields was carried out by a well-trained microscopist for the presence of malaria parasites and density of infection was determined by counting the number of parasites against 200 leucocytes and expressed as parasites/ μ l (assuming that the total white blood cells count is 8000 leucocytes/ μ l). A blood smear was declared as negative (no parasites were detected) after examining 200 thick-blood-smear fields. Microscopist, blinded to the results of ParacheckPf tests, examined all blood smears.

Laboratory confirmed uncomplicated malaria was treated with AS+SP and complicated malaria was treated with quinine according to national protocol for treatment of malaria.

Quality of the work was assured through training in performance and interpretation of the RDT according to manufacturers' instructions, supervision, and all blood smears were rechecked by expert microscopists at Malaria Central Laboratory.

Sensitivity was defined as the number of individuals with a positive ParacheckPf test among the total number of individuals with a positive blood smear. Specificity was defined as the number of individuals with a negative ParacheckPf test among the total number of individuals with a negative blood smear.

Data analysis

Data were recorded and cleaned. Then entered and analysed using Statistical Package for Social Sciences software (SPSS version 10.0). Descriptive analysis was obtained running frequencies for different variables. For comparison of proportion Chi-square test was used and for comparisons of means Mann-Whitney Test was used as the data was abnormally distributed. Test was considered as significant when P-value < 0.05.

Ethical consideration

The study was approved by Federal Ministry of Health, Sudan; and informed consent was obtained from the patients or caregivers of the children included in the study.

Results

Total number of patients included was 164 of them 74 were males and 90 were females, age ranged from 1 month to 74 years (mean age 19 (± 16.5)). *P. falciparum* was determined in 5 (3%) by both microscopy (only asexual stages were detected) and ParacheckPf test, whereas only one (0.6%) of 159 negative on blood smears examination showed positive ParacheckPf test. Mean parasite count of

positive blood smears was 7000 parasite/µl ranged from 3000 to 12000 parasite/µl.

The sensitivity and specificity of ParacheckPf in detecting *P. falciparum* HRP2 were 83.3% and 100%, respectively.

All laboratory-confirmed malaria patients were males and ≥ 5 years of age. History of fever was stated by 140 of the patients, of them 135 had negative blood smears, whereas all microscopically confirmed 5 (100%) had history of fever and out of 6 with ParacheckPf positive reaction 5 (83.3%) stated history of fever. Of the six ParacheckPf positive patients 3 had no past history of malaria where 3 stated that they have experienced malaria before. Of 6 ParacheckPf confirmed malaria 2 were diagnosed as complicated malaria and treated with quinine and 4 patients received Artesunate plus Sulfadoxine-pyrimethamine (AS+S/P).

The study revealed that 99.4% of interviewed patients/caregivers were convinced with the result of ParacheckPf, and only one patient with a negative ParacheckPf test was not convinced with the result of the test (table 1).

Table 1: Microscopic and ParacheckPf Negative and positive results in relation to demographic and clinical characteristics

Characteristics	Total number examined	Microscopy		ParacheckPf	
		Negative	Confirmed	Negative	confirmed
Total	164	159	5	158	6
Sex					
Male	74	69	5	68	6
Female	90	90	0	90	0
Age					
Bellow 5 years	46	46	0	46	0
≥ 5 years	118	113	5	112	6
History of fever					
Yes	140	135	5	135	5
No	24	24	0	23	1
History of malaria					
Yes	123	120	3	120	3
No	41	39	2	38	3
Drug prescribed					
Quinine	2	0	2	0	2
AS+S/P	23	20	3	19	4
Patient convinced with ParacheckPf result					
Yes	163	158	5	157	6
No	1	1	0	1	0

Mean duration of illness was similar between negatives and laboratory confirmed malaria. Mean body temperature among negatives of 37.7 °C was comparable with 38.4 °C and 38.5 °C in

microscopically confirmed and ParacheckPf positive, respectively. The mean of last attack of malaria in months was comparable between negative and microscopy confirmed, negative and

ParacheckPf positive (table 2). However, the mean of last malarial attack (in months) was shorter in laboratory confirmed malaria than negatives,

nevertheless statistical analysis showed comparable result between negative and microscopy confirmed, negative and ParacheckPf positive (table 2).

Table 2. Mean (± SD) duration of illness, axillary temperature and Last attack (in months) in negatives and confirmed malaria by microscopy and RDT (No.164)

Mean (± SD)	Microscopy		P-value*	ParacheckPf		P-value*
	Negative	Confirmed		Negative	Confirmed	
Duration of illness	3.59 (3.96)	4.20 (3.27)	0.31	3.6 (3.97)	4 (2.96)	0.27
Axillary temperature	37.7 (0.99)	38.4 (1.45)	0.21	37.7 (0.99)	38.5 (1.30)	0.11
Last attack in months	8.9 (19.58)	4.4 (5.37)	0.64	9.0 (19.64)	3.7 (5.14)	0.33

* Mann-Whitney Test

Regarding previous treatment for malaria, out of 164 respondents 123 (75%) stated that they received it before, of them 21 (18.6%) was during the last month.

For the 25 patients who received antimalarial drugs the mean (±SD) time from seeing the doctor to the time of receiving treatment was 35 (±7.3) minutes (ranged from 28 to 60 minutes).

Discussion

Microscopic examination of properly prepared and stained blood smears by well-trained microscopist is accurate and reproducible and allows for the identification of the plasmodium species, differentiating between stages and density of infection, moreover microscopy is used for the diagnosis of some other diseases. However, it is not always feasible to sustain accurate microscopy, a situation which is so common in Sudan. Furthermore, there is a lack of practical improvement in the early diagnosis of malaria, in spite of the available technology ⁽¹¹⁾. Therefore, studies addressing the appropriateness of use of RDTs are essential in order to help malaria control programme in Sudan for laboratory confirmation of malaria.

Khartoum state is an area of low malaria transmission and *P. falciparum* is the predominating species together with a relatively high false positive result justifies the introduction of RDT (parasite-based diagnosis) into routine malaria case management ⁽¹²⁾.

The finding that only 6 patients were found to be positive was because Khartoum state is an area of low transmission and data was collected mainly during July a month of low transmission, in addition to the effort directed towards malaria free zone. The finding that less than 4% were found to be infected make statistical analysis a rather descriptive.

In the present study ParacheckPf showed a rather acceptable sensitivity and specificity this similar to previous study ^(13,14), and all ParacheckPf-confirmed malaria had been treated. Moreover, severe/complicated malaria was diagnosed and treated only when laboratory confirmed (i.e. microscopic or/and ParacheckPf positive). This suggests that rapid malaria tests may be an acceptable alternative to poor microscopy for the diagnosis of malaria by clinicians in hospitals and ultimately by health workers in rural areas. One ParacheckPf positive case (with no history of malaria) was found to be microscopy negative is most likely true positive of low parasitaemia ⁽¹⁵⁾.

This study showed that there was reasonably high concordance between microscopy and the ParacheckPf test, moreover, it is faster and requires less training and equipment's confirming previous reports of its suitability as a malaria diagnostic tool ^(16,17).

The finding that 12% ⁽¹⁹⁾ of the patients with negative laboratory tests received antimalarial clearly demonstrated that however there is

reduction in non-evident malaria treatment, nevertheless clinicians continue prescribing antimalarial to non-malarial patients, additionally 18.6% of the patients received antimalarial drugs during the last month, an issue need to be address more effectively by initiating studies to determine the causes of non-malarial fever.

This study does not suggest that this PfHRP-2 based test should replace microscopy as a diagnostic tool at present, but can be used as an alternative or complimentary to microscopy where appropriate.

Due to the inaccuracy encountered microscopic diagnosis of malaria in Sudan, and with the development of rapid diagnostic tests, they may, in the long run, be a realistic alternative to microscopy for the prompt diagnosis of malaria.

In health facilities where the number of examined blood smears is very high or in remote areas the rapid test may be more useful and cost effective than microscopy as time factor is in favour of the rapid test.

However, the cost of the test is of concern, but at present the cost of the test is about 0.5 \$ a price which is rather acceptable peer in mind the true cost due to an incorrect diagnosis which is common in Sudan.

The study provides a clear demonstration of the potential clinical utility of RDTs for the diagnosis of malaria in symptomatic patients⁽¹⁸⁾.

In conclusion, the study showed that ParacheckPf is simple, sensitive, and reliable for detecting falciparum malaria in areas of low transmission, when accurate microscopic diagnosis of malaria in a hospital situation is lacking, or per field situation⁽¹⁹⁾.

The test is acceptable by the patients and the appropriate use of RDT for confirmation of malaria will most likely reduces unnecessary presumptive treatment and self-medication⁽²⁰⁾.

Acknowledgements

We thank the staff of Bashair teaching hospital that helped during data collection. Thanks are extended to Mr. Osama Kubara Senior technician at the hospital and Mr. Tarig El Faki for reexamination of slides. We are grateful for all the patients who agreed to participate in the present study and to the caregivers. The financial support received from National Malaria Control Programme, Sudan is highly appreciated.

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