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Evaluation of the Diagnostic Performance of OptiMAL-IT® Test for the Detection of *Plasmodium falciparum* in South-West Saudi Arabia

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Abstract

Background: OptiMAL-IT® test is a rapid malarial diagnostic test designed to detect at least one protein specific to Plasmodium LDH (pLDH). The diagnostic performance of OptiMAL-IT® was evaluated and compared with the microscopic examinations of thick and thin blood smears, taken as gold standard for the diagnosis of malaria infection.

Methods: A four-year retrospective analysis was done for 238 consecutive suspected patients and 475 apparently healthy blood donors, who were tested for malaria at Rijal Almaa Central Hospital, Asir region, Saudi Arabia between January 2011 and January 2015.

Results: Of the 713 subjects, 74 (10.38%) were confirmed with Plasmodium falciparum infections by cross-checking. All 74 patients (61 patients; 82.4%, and 13 blood donor; 17.6%) were accurately diagnosed with malaria by routine microscopy. 28.15% (67/238) of patients and 3.37% (16/475) of blood donors were positive for malaria by OptiMAL-IT test. OptiMAL-IT® had the following performance indicators for detection of *P. falciparum* among patients, and blood donors enrolled: Sensitivity—98.36% [95% CI (90.02–99.91)], 100% [95% CI (71.66–100)]; Specificity—96.02% [95% CI (91.65–98.25)], 99.35% [95% CI (97.96–99.83)]; Predictive values for positive tests—89.55% [95% CI (79.06–95.34)], 81.25% [95% CI(53.69–95.03%)]; Predictive values for negative tests—99.41% [95% CI (96.27–99.97)], 100% [95% CI (98.97–100)]; Likelihood ratio for positive tests—24.7, 155; Likelihood ratio for negative tests—0.017, 0.00.

Conclusions: The diagnostic performance of OptiMAL-IT malaria test seems to be satisfactory, particularly as a good negative test. OptiMAL-IT can assist in the diagnosis of malaria cases and can be considered for quick screening of blood donor in potentially-endemic settings to prevent transfusion transmitted malaria.

Keywords: Malaria; Rapid; Diagnosis; Performance

Introduction

Malaria has been one of the greatest scourges of humankind since millennia and continues to be a leading cause of mortality and morbidity even in this age of technological and medical advancement [1]. According to the most recent World Health Organization (WHO) Malaria Report (2014), 198 million cases of malaria occurred globally in 2013 and the disease led to 584 000 deaths [2]. Although the burden is heaviest in the WHO African Region [2], and most publications on malaria focus exclusively on sub-Saharan Africa, yet, the large population of South Asia translates into higher risk for more people [1].

Arabian Peninsula is a peninsula of Western Asia situated north-east of Africa. It is the largest peninsula in the world, at 3,237,500 kilometers squared and it demonstrates a high population growth rate; as the result of both very strong inflows of migrant labor, as well as sustained high birth rates [3]. The Kingdom of Saudi Arabia constitutes the largest country in Arabian Peninsula.

The epidemiological aspects of Malaria vary from one region in the Kingdom of Saudi Arabia to another and even within areas of the same region. Epidemiological aspects vary from one year to another, and are affected by the control measures taken against Malaria during that time period [4,5]. The risk of acquisition of malaria in Saudi Arabia is limited to the Southwestern part of the country, with the highest number of cases reported from Jizan and Asir regions [6]. In these two regions, there are still 29 active foci for malaria acquisition. According to WHO country report of Malaria 2014, the total confirmed cases in Saudi Arabia was 2513 cases [7].

Malaria is caused by parasites of the *Plasmodium* family and transmitted by female *Anopheles* mosquitoes. There are four different human malaria species (*P. falciparum, P. vivax, P. malariae* and *P. ovale*). While *P. falciparum* and *P. vivax* are the most prevalent worldwide, *P. falciparum* is the most dangerous [8]. Importantly, *P. falciparum* is the main malaria species in Saudi Arabia with hundred percent prevalence among malaria cases [7].

With ambitious new targets set to reduce the global burden of malaria, we must urgently optimize the current tools for disease control, as well as optimizing and reevaluating our current diagnostic tools [9]. In most countries microscopy has largely remained a tool limited to reference laboratories and hospitals [10]. Microscopy is regarded as the 'gold standard' for malaria diagnosis [11].

However, the lack of skilled technologists in medical facilities in affected areas may lead to poor interpretation of data [12]. This led to the development of rapid diagnostic test (RDT) for malaria, which is a device that detects malaria antigen in a small volume of blood by immune-chromatographic assay with monoclonal antibodies directed against the target antigen and impregnated on a test strip. OptiMAL-IT® is an immuno-chromatographic test, using monoclonal antibodies against the metabolic enzyme parasite lactate dehydrogenase (pLDH) of *Plasmodium* species [13].

Rijal Almaa Hospital is a central hospital at Asir region, serving Rijal Almaa government (population: 66,000), and it represents a referral for many health centers around. OptiMAL-IT® test is used along with microscopy for the diagnosis of endemic malaria cases and for screening for blood transfusion. The results for both microscopy and OptiMAL-IT® are recorded and the thick and thin blood smears itself are kept; regardless it is positive or negative for malaria.

Recently, few discrepancies between the results of blood smears by microscopy, and that of OptiMAL-IT® test were noted. However, the lack of a hematopathologist (or even a clinical pathologist) in this big hospital during the last two years necessitated the review and check of all recorded blood smears and also the evaluation and validation of OptiMAL-IT® test results. Thus, the aim of this retrospective study is to evaluate the OptiMAL-IT® Test, based on plasmodium lactate dehydrogenase for malaria detection in the south-west of Saudi Arabia and compare the results of microscopic examination of thick and thin blood smears as the gold standard.

Subjects and Methods

Files of all adult patients attended the outpatient clinic and the emergency ward of Rijal Almaa Central Hospital between January 2011 and January 2015, who underwent a malaria test, were reviewed retrospectively. In general, physician-identified patients who presented with symptoms consistent with malaria [history of fever with or without chills (axillary temperature [37.5°C), sweating, and headache] and referred to the laboratory for a malaria test.

As part of routine health-service delivery in Rijal Almaa Hospital, patients with clinical symptoms suspicious of malaria were to undergo first an RDT. The thin and thick blood smears and microscopic examination were then performed within three hours and during midnight. Demographic information and details on OptiMAL-IT test result and microscopy result were extracted from patient's file and laboratory database.

OptiMAL-IT test protocol:

Venous blood was drawn into EDTA-coated syringes, distributed into sterile test tubes, and placed immediately on ice. Additional samples comprised blood specimens referred secondarily from other health centers for confirmation. Routinely, OptiMAL-IT detection tests were performed according to manufacturer's instructions in parallel with thin and thick film microscopy on all specimens by separate operators blinded to the results of the other assays.

OptiMAL-IT (Diamed, Cressier, Switzerland) is an immuno-chromatographic test, using monoclonal antibodies (Mabs) against the metabolic enzyme pLDH (parasite lactate dehydrogenase) of *Plasmodium*. These Mabs are classified in two groups; one specific for *Plasmodium falciparum* and the other is a pan-specific Mab, which reacts with for all four species of human *Plasmodium*; *P. falciparum, P. vivax, P. ovale,* and *P. malariae*.

Interpretation of the assay test strip results was done as; 1) When one control band and two test bands appeared, the test was considered to be positive for *P. falciparum*; 2) When one control band and one test band appeared, the test was considered positive for *P. vivax*; 3) When only one control band appeared at the top of the test strip without test band, the test was considered to be negative. All blood samples were tested with the OptiMAL-IT test according to manufacturer's instructions.

Validation and Re-evaluation using Microscopy

Thick and thin blood smears were re-examined (blind examination) for *Plasmodium* infections without reference to results of microscopy and OptiMAL-IT assay. This blind examination was compared with the previous recorded results of microscopy and OptiMAL-IT assays.

Statistical analysis

All data were recorded in Excel 2000 software (Microsoft Corp., Redmond, WA, USA). Specimens were classified as true positive, true negative, false positive, or false negative for each test under evaluation compared with validation microscopy. The following performance indices were calculated: Sensitivity, specificity, positive predictive values (PPV) for positive and negative predictive values (NPV) for negative tests, and Likelihood ratios for positive and negative tests.

Results

Between January 2011 and January 2015, a total of 253 patients and 475 random apparently healthy blood donors were tested for malaria (as per laboratory records). 15 patients were excluded because their clinical files could not be retrieved. Thus, a total of 713 subjects comprising 238 feverish patients and 475 consecutive apparently healthy blood donors, were included in this study.

Table 1 displays the differential diagnosis obtained by OptiMAL-IT and the results of primary field microscopy at the hematology division of Rijal Almaa Hospital Laboratory. No discrepancies were observed between the primary routine microscopy and the validation microscopy results from the research laboratory.

Table 2 shows the comparative performance of OptiMAL-IT detection assay calculated on the basis of our confirmed microscopy results. Overall, 25.634% (61/238) of patients and 2.73% (13/475) of blood donors enrolled to the study were diagnosed with malaria by field microscopy, whereas 28.15% (67/238) of patients and 3.37% (16/475) of blood donors were positive for malaria by the rapid OptiMAL-IT test (total 10 false positives, and 1 false negative) (Table 1).

Using OptiMAL-IT, *P. falciparum* diagnosis among blood donors showed a higher proportion of false positives, 18.75%, compared to that of patients, 10.45%. Whereas, false negatives amongst patients diagnosed of malaria by OptiMAL-IT was higher, 0.59%, compared to that of blood donors, 0.00%.

On the other side, OptiMAL-IT demonstrated a slightly lower sensitivity for detection of *P. falciparum* in patients, 98.36% [95% CI (90.02–99.91)], compared to the blood donors, 100% [95% CI (71.66–100)]. Overall, the sensitivity of OptiMALIT was 98.65% [95% CI (91.68–99.93)], with a specificity and positive predictive value of

98.43% [95% CI (97.04–99.20)], and 87.96% [95% CI (78.51–93.76)], respectively (Table 2).

The OptiMAL-IT had the following performance indicators for detection of *P. falciparum* among patients and blood donors respectively: Specificity—96.02, 99.35%; Predictive values for positive tests—89.55, 81.25%; Predictive values for negative tests—99.41, 100%; Likelihood ratio for positive tests- 24.73, 155; Likelihood ratio for negative tests—0.017, 0.00 (Table 2).

OptiMAL-IT	Result of Microscopy[a] (number of samples)			
	Pf +ve	Non-Pf	Gametocytes	Negative Tests
Pf[b] only				
Patients (n=67)	60	0	6	7
Blood Donors (n=16)	13	0	0	3
Non-Pf				
Patients (n=0)	0	0	0	0
Blood Donors (n=0)	0	0	0	0
Negative				
Patients (n=170)	1	0	1	169
Blood Donors (n=459)	0	0	0	459

[a]There were no discrepancies between primary and cross checking microscopy readings

[b]All confirmed positive malaria cases were with Plasmodium falciparum (Pf)

Table 1: Differential diagnosis of malaria cases by OptiMAL-IT rapid antigen testing versus Microscopy.

	Patients			Blood Donors			Total Subjects		
	Estimated Value	95% Confidence Interval		Estimated Value	95% Confidence Interval		Estimated Value	95% Confidence Interval	
		Lower Limit	Upper Limit		Lower Limit	Upper Limit		Lower Limit	Upper Limit
Prevalence	25.74	20.4	31.88	2.72	1.52	4.73	10.39	8.3	12.93
Sensitivity	98.36	90.02	99.91	100	71.66	100	98.65	91.68	99.93
Specificity	96.02	91.65	98.25	99.35	97.96	99.83	98.43	97.04	99.2
Positive	28.27	22.72	34.53	3.35	1.99	5.49	11.66	9.44	14.3
Negative	71.73	65.47	77.28	96.65	94.5	98.01	88.34	85.7	90.56
Positive Predictive Value (True Positive)	89.55	79.06	95.34	81.25	53.69	95.03	87.96	78.51	93.76
False Positive	10.45	4.66	20.94	18.75	4.97	46.31	12.05	6.24	21.49
Negative Predictive Value (True Negative)	99.41	96.27	99.97	100	98.97	100	99.84	98.97	99.99
False Negative	0.59	0.03	3.73	0	0	1.03	0.16	0.01	1.03
likelihood Ratios:									
Positive [C]	24.73	11.958	51.146	155	50.174	478.833	62.938	34.01	116.471
Negative [C]	0.017	0.002	0.119	0	0	NaN*	0.014	0.001	0.096
Positive [W]	8.571	4.232	17.361	4.333	1.521	12.344	7.3	4.06	13.126

Negative [W]	0.006	0.0008	0.042	0	0	NaN*	0.002	0.0002	0.011
[C] = conventional [W] = weighted by prevalence *'NaN' in any of the above cells means that the calculation cannot be performed because the values entered include one or more instances of zero.									

Table 2: Diagnostic Performance of OptiMAL-IT in patients, blood donors, and total subjects calculated on the basis of confirmed microscopy results.

Discussion

For efficient treatment and management of malaria, rapid and accurate diagnostic testing is imperative. The cornerstone of malaria diagnosis in the laboratory is microscopy. Microscopy is inexpensive and sensitive when used appropriately [12]. Moreover, it can be used to differentiate species and to follow treatment [14,15]. However, this requires a well-maintained microscope, standard staining procedures and a good expertise in microscopy. These conditions are not always easy to meet in the limited resource health centers. Furthermore, microscopy may be time consuming and labor intensive [12], cannot detect sequestered *P. falciparum* parasites [16], and it is less reliable at low-density parasitaemia [17].

In view of these limitations of malaria diagnosis using microscopy, there is a need for a simple-to-perform rapid test for the diagnosis of malaria. Malaria rapid diagnostic tests (RDTs) are increasingly used, especially in non-endemic settings. OptiMAL test is a rapid malaria detection test (RDT) based on pLDH [13], which is an enzyme in the glycolytic pathway of the *Plasmodium spp.,* and is produced by sexual and asexual stages of the parasite. Differentiation of malaria parasites is based on antigenic differences between the pLDH isoforms. Since pLDH is produced only by live *Plasmodium* parasites, this test can differentiate live organisms from dead ones [18].

Several investigations have reported on the performance of OptiMAL-IT assays in various geographical regions [19-26]. Although the efficiency of OptiMAL-IT test may vary from one geographical location to the other, no systematic survey on the performance of OptiMAL-IT has been conducted in Saudi Arabia, and the extent of its potential application remains unclear. In this study, we have evaluated the diagnostic performance of OptiMAL-IT, against conventional microscopy on a population of moderate risk for malaria, in Rijal Almaa District, Asir region of Saudi Arabia. Provided cross-checking microscopy was accurately read for all smears of all patients and blood donors. Our results revealed that OptiMAL-IT test showed good performance for malaria diagnosis under routine conditions for patients and blood donors. The sensitivity of the OptiMAL-IT assay for detection of *P. falciparum* in blood donors was 100%, higher than that observed for patients (98.36%). Additionally, the specificity of the OptiMAL-IT assay for detection of *P. falciparum* in blood donors was 99.35 %, higher than that observed for patients (96.02%). Moreover, the negative predictive value of the OptiMAL-IT assay for detection of *P. falciparum* in blood donors was 100 %, slightly higher than that observed for patients (99.41%). On the other side, the positive predictive value of the OptiMAL-IT assay for detection of *P. falciparum* in patients was 89.55%, higher than that observed for blood donors (81.25%).

According to the WHO (1999), these methods must be benchmarked against microscopy to be validated. For example, RDTs must have a minimum sensitivity of 95%, compared with microscopy, and a minimum specificity of at least 90% [11]. Notably, these diagnostic performance standards have been met in our results for OptiMAL-IT assay for detection of P. falciparum.

Several conflicting studies have previously reported trials of OptiMAL for the diagnosis of malaria in different countries. Our findings are similar to some studies reported more than 90% specificity and sensitivity for Optimal-IT [19-22]. In contrast, less than 90% specificity and sensitivity for Optimal-IT were recorded in other studies [23-25]. Alternatively, low sensitivity (ranging from 63 to 94%) and specificities (ranging from 97 to 100%) of the OptiMAL diagnostic test were shown by several reports from Afghanistan, Honduras, Kuwait, Turkey, France, and Peru [26-31]. Other studies from Canada [32] showed a sensitivity of 29% but a specificity of 95.6%. In a study done in Congo, the sensitivity of OptiMAL did not reach the acceptable threshold of 90% whereas the specificity was 97% [33].

Transfusion-transmitted malaria can be a major problem in blood banks. Semi-immune individuals with low level of parasitemia may remain asymptomatic [34-36]. In addition; malaria parasites have the ability to survive in the storage conditions (4°C) of donated blood [37]. In this study, we evaluated the possibility of using Optimal-IT as a screening method for *Plasmodium* parasites in blood banking. OptiMAL-IT method may be a simple, rapid (20 minutes) and sensitive diagnostic test for malaria. The sensitivity of this test was very close to that obtained by microscopic examination, and it did not require highly skilled personnel to perform or interpret.

RDTs are very useful in endemic areas for mass screening in a short time. Additionally, OptiMAL-IT test can detect all four human *Plasmodium* species through detecting the pLDH enzyme produced only by living parasites [38]. On the other side, the major disadvantage of OptiMAL-IT is decreased sensitivity at low parasitaemias. The sensitivity of malaria screening by microscopic examination limit is about 50 parasites/μL and that for RDT is about 100 parasites/μL. In addition, RDT cannot differentiate between *P. falciparum* and mixed infection [39,40].

False-positive results, representing 12.1% in our study may be due to either cross infections (e.g. dengue, hepatitis C, toxoplasmosis, tuberculosis, *Salmonella typhi*) [41-43], or cross-reactivity due to self-antibodies such as rheumatoid factor and anti-nuclear antibodies) [44,45]. False-negative results may be due to the prozone effect [46], or may be from patients treated with antimalarial drugs before their inclusion in the study, so that dead parasites were observed on microscopy, but pLDH production could have been halted by therapy [47,48].

Two limitations may be addressed in this study. Firstly, being a retrospective study, we could not control exposure or outcome assessment. Secondly, the choice of blood smears as the reference diagnostic test, instead of Molecular techniques, may appear a limitation of our study [49]. Moreover, it may be advocated to correlates the microscopy results with molecular diagnostic techniques. It is established that molecular techniques are the most

accurate methods that can detect low parasitemia and mixed infection, however, also laborious and costly. Additionally, clearance of plasmodium DNA from the bloodstream following antimalarial treatment should be studied to support and validate this option [50]. Further, polymerase chain reaction (PCR) contamination was explored as a potential confounding factor in the association between transmission intensity and apparent microscopy sensitivity [51].

Conclusion

Saudi Arabia is now one of the 32 countries that are challenging malaria elimination. Thus, it is mandatory to establish its malaria surveillance program. This can be achieved by the integration of case detection and proper treatment to map and target areas of transmission and track any outbreaks [52]. As a conclusion, this study shows that *P. falciparum* is the primary species of malaria in Rijal Almaa District, Asir region, KSA. Optimal-IT® is a technically powerful test to effectively discriminate patients with and without malaria. Our results suggest that OptiMAL-IT may serve as a good negative test for blood donors screening and aids in the diagnosis, hand with hand, with microscopy for diagnosis of malaria.

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Author's Note

All interventions were part of standard healthcare practices and thus ethical approval was neither obliged nor sought.

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