

EVALUATION OF IMMUNOCHROMATOGRAPHIC TEST FOR DIAGNOSIS OF FALCIPARUM MALARIA

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ABSTRACT

This study has evaluated Immunochromatographic test (ICT) as a new diagnostic technique, recently introduced in the Sudan for the detection of *Plasmodium falciparum* (*P. falciparum*) parasite. A total of one hundred blood samples were examined using ICT in comparison with the traditional microscopic method. All samples were found to be positive for *P. falciparum* when stained by Giemsa stain and examined microscopically. The result revealed 98 asexual stages and 2 sexual stages of *P. falciparum* were observed. However, 98 of these blood samples were found to be positive for *P. falciparum* when examined by the ICT. On the other hand, statistical analysis showed a high degree of sensitivity and specificity of the ICT ($P<0.05$) compared to microscopical method utilized traditionally in detection of malaria parasites in patients blood.

KEY WORDS: Malaria, Immunochromatographic test (ICT), Giemsa stain, *Plasmodium falciparum*, sexual stages, asexual stages.

ملخص:

أجريت هذه الدراسة لتقييم اختبار المناعة الكروماتوغرافي كتقنية حديثة استخدمت في السودان لتشخيص طفيل بلازموديوم فالسيبرم. تم فحص مائة عينة دم باستخدام اختبار المناعة الكروماتوغرافي ومقارنتها بالفحص الميكروسكوبي. جميع العينات كانت ايجابية لبلازموديوم فالسيبرم عند صبغها بطريقة جيمسا العيارية وفحصها مجهريا وقد كانت النتيجة ثمانية وتسعون طور لاجنسي واثنين طور جنسي , مقارنة بثمانية وتسعون عينة من هذه العينات كانت موجبة عند استخدام اختبار المناعة الكروماتوغرافي. اظهر التحليل الاحصائي حساسية ونوعية عالية لاختبار المناعة الكروماتوغرافي ($P<0.05$) عند مقارنته بالطرق المجهرية المستخدمة تقليديا لاكتشاف طفيل الملاريا في دم المرضى.

INTRODUCTION

Malaria continues to be a major killer of mankind, especially in developing countries. It is most common in tropical and subtropical regions of the world (Marquart, *et al.*, 2000). According to World Health Organization (WHO) more than 40% of the world's population are exposed to the risk of malaria (WHO, 1993). Out of these, 270 Million suffer from malaria, and more than one million die of the disease every year. It is defined as an acute disease caused by malarial plasmodia transmitted by mosquitoes of the genus anopheles. This illness is characterized by a cyclic course with period of acute febrile attacks and paroxysm-free intervals as well as splenohepatomegally, anemia and occasional severe lesions of the nervous system, kidney and other organs (Loban and Polozok, 1989). A key feature of WHO new malaria control is a rapid diagnosis even at village level (WHO, 1995).

Globally, malaria control strategy is principally based on early diagnosis and prompt treatment. Thus, delayed diagnosis and treatment of patients especially, with *P. falciparum* infection may lead to development of severe and complicated malaria, that consequently cause a high mortality rate (Francisco, *et al.*, 1990).

The microscopic diagnosis of malaria is the principal method used for confirming the diagnosis of malaria, but the method has some limitations particularly in many developing countries such as the lack of adequate laboratory facilities and well-trained personnel.

In the Sudan, microscopic diagnosis of malaria was found to be unreliable due to several factors, including the general condition of the microscope, type and quality of stains, and the proficiency of the technicians (Adam, 2001). This work aimed at evaluation of ICT as new diagnostic tool for malaria.

MATERIALS AND METHODS

Patients

Febrile patients, presenting with symptoms of malaria, who visited Abayazid Hospital, Khartoum State were enrolled in the study.

Using finger punches blood samples were collected from 100 febrile patients (1-70 years old) to prepare blood films. Twenty samples were also collected from healthy subjects to be used as negative control. Blood specimens for ICT test were collected similarly from patients and healthy subjects in

capillary tubes. Blood films were prepared and stained by Giemsa 10 (B.D.H. Co. Ltd.) according to Cheesbrough, (1998).

Examination of blood films

The slides were examined using a light microscope and immersion oil lens. The number of parasites was counted and reported according to Cheesbrough, (1998).

ICT Test

The ICT test was carried out for positive blood films and performed as instructed by manufacturer (MakroMed Co. Ltd).

Interpretation of the test:

- i- Negative: Only one red line appear in the (C) window of the test.
- ii- Positive (*Falciparum malaria*): Two red lines in the (C) window and on the (T) window were visible on the test.
- iii- The test was considered invalid, if the control line does not appear.

The sensitivity (i.e.the ability of the test to detect the smallest amount of antigen in the test sample), was calculated by the following formula:

$$\frac{\text{True positive}}{\text{True positive} + \text{false negative}} \times 100$$

Whereas specificity (i.e. the ability of the test to detect the entire positive and the entire negative), was calculated according to following formula:

$$\frac{\text{True negative}}{\text{True negative} + \text{false Positive}} \times 100$$

Data analysis

Chi square test statistical analysis was performed to compare the sensitivity and specificity of ICT with reference to the standard Giemsa staining method commonly used for diagnosis of malaria in Sudan.

RESULTS

The study examined a total of 100 blood samples collected from febrile patients. Samples were examined by two techniques. These are, microscopic

examination of stained blood films using Giemsa stain and Immunochromatographic test (ICT).

Microscopical examination

Using Giemsa stain a total of 100 (100%) blood films (thick & thin) stained by Giemsa stain were found to be positive. Asexual stages were detected in 98 (98%) samples, while sexual stages (gametocytes) were detected in 2 (2%) samples only.

Immunochromatographic test ICT

Ninety eight (98%) of malaria positive slides, were also ICT positive, two (2%) of hundred malaria positive slides were ICT negative, they show only gametocyte without ring stage, (Table 1).

Table 1. Comparison between Giemsa stain & ICT

Result	Stage <i>P. falciparum</i>	
	Sexual	asexual
Positive*	2	98
Negative*	0	0
Total	2	98

* Positive indicates the presence of *P. falciparum* in blood or its antigen.

* Negative indicates neither *P. falciparum* detected nor its antigen.

Sensitivity and specificity

As shown in table 2 the sensitivity of the ICT to detect *P. falciparum* compared to microscopic was 98% and the specificity was 100%. Chi-Squire test showed significant results (0.00) which are less than 0.01 that means the results are highly significant in all levels (Table 3).

Table 2. Sensitivity and specificity of the ICT compare to microscopic examination of blood films stained by Giemsa stain.

	Immunochromatographic test ICT
Sensitivity	98%
Specificity	100%

Table 3. Chi-Square Tests

Test	Value	Df*	Asymp.Sig. (2-Sided)
Pears chi-Square	24.024	3	.000

* Df = degree of freedom

DISCUSSION

The present study was conducted to evaluate the ICT used for diagnosis of malaria in comparison to the classical microscopic techniques utilized in

diagnosis of malaria in Sudanese patients and then to point out the accurate technique leading to a reliable result. The microscopic diagnosis was used as standard method to which other new method, Immunochromatographic test (ICT) was compared.

This study was carried out on 100 patients clinically suspected to have malaria admitted to Abayazid Hospital.

Previous studies carried out by Dowling & Shute (1996) and Spielmen, *et al.*, (1988), recommended that the use of Quantitative Buffy Coat (Q.B.C) test for diagnosis of malaria instead of microscope.

To evaluate the use of ICT test for the diagnosis of *P. falciparum* malaria, a study was carried out in 13 patients who presented with fever and *P. falciparum* was detected microscopically and in 17 healthy individuals without any recent history of fever and were microscopically negative for *P. falciparum*, the result of ICT test showed that in 13 microscopically positive case the ICT was also positive and the 17 microscopically negative cases were also negative by the test. In spite of the small study sample size it appears that ICT test is sensitive in detecting *P. falciparum* infection and the procedure is easy and simple to perform (Zayed and Dafalla, 1997).

In central India, a study was undertaken to assess the performance and usefulness of ICT test as diagnostic method in highly malarial, inaccessible forest village 353 patients with fever were scanned by the ICT test in parallel with thick blood film examination, the sensitivity and specificity were 100% and 84.5% respectively (Singh, *et al.*, 1997).

A field study conducted to assess of ICT based on detection of *P. falciparum* HRP-2 in peripheral blood showed, that in 173 patients the sensitivity and specificity were 98.5% and 97.1% respectively (Valecha, *et al.*, 1998). It is a rapid test that detects the presence of HRP-2 in whole blood. Field tests conducted in Solomon Island the test to have a high sensitivity (100%) and specificity of 96.2%, when compared with light microscopy (Garcia, *et al.*, 1996).

Howard *et al.*, (1986), showed that *P. falciparum* HRP-2 is the one of the Histidine rich proteins synthesized by *P. falciparum* and released from infected erythrocytes as a water-soluble protein. It can be detected in 2-8

hours after ring development indicating that it is actively secreted from infected cells. The amount of HRP-2 release continuous to increase through out the erythrocytes cycle with a large amount being released during schizont rupture. *P. falciparum* HRP-2 was found to be localized in the parasite cytoplasm and infected erythrocytes membrane (Howard, *et al.*, 1986).

Detection of HRP-2 antigens is performed by IgG monoclonal antibodies, which are, prepared against these antigen (WHO, 1996).

Evaluation of the rapid test ICT in the diagnosis of falciparum malaria infection in comparison to microscopic examination, showed that the test is 100% specific, but it does not detect the mature gametocytes of *P. falciparum* the sensitivity of the test appears to be approximately 93%.

The ICT for *P. falciparum* has been shown to give a constant specificity and sensitivity of in the region of 90% compared to standard thick and thin blood film examination (WHO, 1996).

ICT method for detection of *P. falciparum* HRP-2 with limits of detection equal to or better than those provided by light microscopy, with matching specificity and sensitivity of around 90%(Kodisinghe, *et al.*, 1997).

The results obtained during this study, revealed that the microscopical examination of blood films stained by Giemsa stain has high sensitivity and specificity of 100%. The results were in agreement with Van, *et al.*, (1998) and Ross (1903) whom reported that the ICT could not replace the conventional microscopic method in the diagnosis of malaria parasite. They showed that the thick and thin blood films examination are more sensitive and specific. Moreover, it allows distinguishing between different species of malaria parasite, but require prolong time.

In the present study the sensitivity of the ICT was found to be 98% compared to microscopic examination of blood films. This result was similar to Valecha *et al.*, (1998).

In previous studies carried by Shift, *et al.*, (1993) found that the sensitivity and specificity of ICT 88.9% & 87.5%, Beadle, *et al.*; (1994), 96.5% & 98%, Banchougaskorn, *et al.*; (1996) 98% & 96% and Kodisinghe, *et al.*; (1997), 90.2% & 99.2% respectively.

In this study the sensitivity of ICT 98% which was similar to those studies carried by Shift, *et al.*, (1993), Beadle, *et al.*; (1994), and Banchouga- sksorn, *etal.*; (1996).

The specificity was found to be 100% which was similar to Beadle, *et al.*; (1994) and Kodinsinghe, *et al.*, (1997). However, the ICT test failed to detect falciparum gametes in two samples (2%). This finding was similar to that reported in Indian Pediatrics (Nharakunwa and Shiff, 1997). It was likely due to the absence of circulating Histidine Rich Protein2 in blood which produce by asexual stages.

The ICT has disadvantage in diagnosing partial response of treatment because the extent of antigenemia may not be detected by the test results unless the test is quantitative. Also Histidine Rich Protien-2 is known to circulate for a definitive period of time (estimate 1-2 weeks) following elimination of peripheral parasitaemias (Namsiripongqun, *et al.*, 1993). Thus, persistence of antigens after elimination of viable parasites from the peripheral blood may explain some of the false positive results.

The average time to prepare one ICT test was found to be only 5 minutes, while the average time to prepare, stain (Giemsa) and examine blood films was 30 minutes. The time to do slide preparation is even prolonged; negative slides require an extremely long time to rule out malaria infection. This at time makes microscopy very tedious, laborious and lowers the efficiency in situation where a large number of smears have to be examined. This means that in *P. falciparum* infection the time to make an accurate diagnosis by microscopy is sufficient to make an accurate diagnosis using ICT test and gain 25 minutes of working time

The ICT was easily operable, the operator needed only one demonstration to learn the technique.

At the time of the present study, the cost of each ICT *P. falciparum* was estimated at 15 SDG. The cost of microscopic examination was estimated at 5.0 SDG in private laboratory, while in the hospital 3.0 SDG. Although the cost of ICT test is higher than that of microscopical examination, no influence of age or sex in the study group was noticed.

The study, utilized Giemsa stain for staining, blood smear gaves well stained smear, that encouraged the microscopist to an attentive review of blood elements, an essential of accurate malaria diagnosis, it gave the correct staining of malaria parasite i.e chromatin stained purple red and

cytoplasm stained blue and all smear stained homogeneously, the sensitivity and specificity were found to be 100%.

On the other hand, Field stain A & B gave good color contrast in thick blood films, it gave a good color balance between blue and red, i.e chromatin dark red and cytoplasm stained blue. The result showed 98 samples (98%) out of hundred were positive. Moreover, using Field stain A only, showed a marked decrease in reliability of results. The cytoplasm and chromatin stained blue, 97 samples (97%) were positive.

The study concluded that the ICT is reliable, sensitive and specific ($P < 0.05$) in detection of *P. falciparum*.

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