Original Article

Validity of Rapid Diagnostic Test compared to Light Microscopy for Malaria Diagnosis at Amana Clinic in Khartoum State, 2020

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Abstract

Background: Early diagnosis and proper management are important issues in malaria control. Since malaria is a serious disease and highly endemic in Sudan, there is a need to identify the best diagnostic tools to help in the proper management and control of the disease. Rapid diagnostic tests (RDTs) and microscopy are routinely used for the diagnosis of malaria in Sudan. This study was conducted to assess the validity of RDTs of malaria compared to microscopy, using sensitivity and specificity of the RDTs against the standard method (Light Microscopy).

Materials and Methods: One hundred patients with a clinical diagnosis of malaria attending Amanah Clinic Laboratories were tested by two methods: (a) Peripheral thick and thin blood films for the light microscopy diagnosis and (b) immunochromatographic test (ICT) for Malaria as a RDT. The results were compared using the sensitivity and specificity of the ICT against light microscopy.

Results: The ICT detected Plasmodium species infection with a sensitivity of 89%, specificity of 86%, positive predictive value was 99% and negative predictive value was 31.7%.

Conclusion: Our study shows that diagnostic accuracy of RDT was suboptimal compared to the gold standard method of microscopy.

However; RDT is a valid diagnostic test for the majority of malaria cases, because it's quick and easy to use. In difficult cases, microscopy in expert hands remains the gold standard method and the final court of appeal

Keywords: *Malaria Diagnosis, Microscopy, ICT, Validity, Sensitivity, Specificity, Predictive values.*

Introduction:

Malaria is a serious and sometimes fatal disease caused by a parasite that commonly infects a mosquito species that feeds on humans. Malaria is considered the most severe public health problem worldwide. (1) Nearly half of the World's population lives in areas at risk of malaria transmission in about 87 countries as per the 2021 World Malaria Report. In that report, 241 million clinical episodes of malaria were estimated in the year 2020. The World Health Organization (WHO) African Region report estimated the African continent malaria prevalence as 95% and the death toll to be 627,000 deaths. (1)

The African Region continues to carry a disproportionately high share of the global malaria burden. In 2019, six countries accounted for approximately half of all malaria deaths worldwide: Nigeria (23%), the Democratic Republic of the Congo (11%), the United Republic of Tanzania (5%), and (4%) for each of Burkina Faso, Mozambique and Niger. (2) In Sudan, over one million malaria cases were reported across the country in the first half of 2022. The most vulnerable groups were travelers and migrants from areas with low or no malaria infections, , pregnant women, especially during the first two trimesters of pregnancy and children under five.(3)

People who get malaria are typically very sick with high fever, shaking chills, and flu-like illness. Although malaria can be a deadly disease, illness and death from malaria can usually be prevented. (4) A rapid and accurate diagnosis is the first step in the malaria effective management. (5) Early diagnosis and treatment of malaria reduces disease and prevents death. It also contributes to reducing malaria transmission. The best available treatment, particularly for *Plasmodium falciparum* malaria, is artemisinin-based combination therapy (ACT). (6) Light microscopy and Rapid Diagnostic Tests (RDTs) are currently considered the two diagnostic procedures with the highest impact on controlling malaria. Microscopy is a valuable diagnostic tool, and in expert hands, it can detect up to 50 parasites/µl of blood. (7) Immunochromatographic test (ICT) is a commercially available malaria Plasmodium/pan rapid test, which allows the detection of malaria antigens. It is a qualitative membrane-bound assay for *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae* detection. The test membrane is pre-coated with anti-Histidin Rich Protein-2 (anti-HRP-2) specific antibodies for *Plasmodium falciparum* and Plasmodium specific anti-aldolase antibodies for the detection of the other three species of Plasmodia. (7) In Sudan, like many other African countries, malaria is highly prevalent in areas where health recourses are often poor and expert health personnel are scarce. Malaria diagnosis by quality microscopy is often not available in such areas. ICT is a RDT that is not operator dependent and is a welcome addition to the diagnostic armaments of malaria. This study aims to test the diagnostic accuracy of ICT compared to the standard microscopy operated by expert personnel in malaria diagnosis.

Materials and Methods:

A descriptive cross-sectional study was conducted at Yathrib area in Khartoum State from August 2019 to August 2020.

The study was carried out in the Amanah Clinic. This center was established in the year 2018 and is equipped with a modern laboratory, run by qualified personnel.

Study population:

Patients referred to the Amana Clinic laboratory during the study period requesting laboratory diagnosis of malaria were considered for the study. Critically ill patients were excluded. Patients who agreed to participate in the study after informed consent were included.

Sample Size:

The sample size was determined according to the following formula**:**

$$n=N/ [1+N(E^2)]$$

Where **n** is the sample size; **N** is the population of the study and **E** is the margin of error (0.05) indicated by the 95% C.I.

The population of the study was N=134 patients. Using the above formula, **n** was computed to be 100 patients. They were selected by simple random sampling technique. Peripheral thick and thin blood films for the microscopy diagnosis and a rapid ICT for Malaria were performed on the study group.

Procedures for thin and thick blood films and RDTs preparation:

The skin of the index finger was sterilized using a 70% alcohol swab and pricked with a sterilized lancet. Capillary blood sample was obtained for the thin and thick blood films as well as the ICT for malaria diagnosis.

(a) Thin blood film preparation: A small drop of blood was placed on a pre-cleaned labeled slide. Another slide (spreader) was held at a 30-45° angle and used to spread the blood along the contact line up to the end of the lower slide. The thickness of the blood smear decreased progressively towards the feathered edge of the slide, thus forming cells in a monolayer.

The thin smear was allowed to dry, and then fixed by dipping in absolute methanol for two seconds.

(b) Thick blood film preparation:

A thick blood film consists of a thick layer of lysed red blood cells. To prepare such a film, three small drops of blood were placed in the center of a pre-cleaned labeled slide. The corner of another slide was used to spread the drops to form a circular pattern of 2-3cm in diameter. The slide was laid flat and the smear was allowed to dry thoroughly and protected from dust, fluid and insects. To accelerate the drying of a smear a fan was used. After that Geimsa stain was used to stain the smears. (8)

(c) Principles and preparation of RDTs, also known as ICT:

Malaria RDTs are qualitative immunochromatographic "lateral flow" tests. They are prepared in dipstick (strip), cassette or card forms to detect malaria antigens in the peripheral blood. The term "lateral flow" refers to the migration of liquid across the surface of a nitrocellulose membrane. Malaria antigen from a lysed blood sample is reacted with anti-malaria monoclonal antibody conjugated to colloidal gold (pink-mauve) particles. The antigen-antibody colloidal gold complex migrates along the nitrocellulose membrane, where it becomes bound (captured) by a line of specific monoclonal antibodies, producing a pink line in the test result area. This line can be seen after a washing buffer has removed the background hemoglobin. A further pink line (i.e. inbuilt positive control) is produced above the test line, indicating that the test reagents have migrated satisfactorily (it is not a malaria antigen control). (8) The Kits used in the study were manufactured by SD BIOSENSOR company from Republic of Korea.

Interpretation of malaria diagnostic tests:

(a) Interpretation of microscopic examination: In this study, malaria diagnosis was considered positive if the parasite trophozoite stage was detected.

Simple criteria were used to differentiate between the two species of malaria. For the *P.falciparum*, the trophozoites were small to medium in size. Many of them had ring and comma shapes. *Chromatin dots* (often *two dots*) and regular cytoplasm were observed.

For *P.vivax* the trophozoites were large in size and had few to moderate numbers of broken rings. The most common shapes were irregular forms. The trophozoite contained a *single chromatin dot*.

(b) Interpretation of ICT: The test results were interpreted as follows;

- Test considered *P. falciparum* positive if one line appeared in the control region and one in the *P. falciparum* specific region.
- Test considered as non-falciparum Plasmodium-species-specific positive if one line appeared in the control region and one in the pan malarial region.
- Test considered to be mixed infection if one line appeared in the control region, one in the *P. falciparum* region and one in the pan malarial region.
- Test considered to be negative if one line appeared only in the control region. (8)

Data Analysis:

Data were analysed using statistical package for the social sciences (SPSS) version 20. Matthews Correlation Coefficient (MCC) was used to calculate the ICT sensitivity and specificity.

Validity of a test is defined as its ability to distinguish between who has a disease and who does not. (9, 10) Validity has two components: sensitivity and specificity. (9, 10) **Sensitivity** is defined as the ability of the test to identify correctly those who have the disease. **Specificity** is defined as the ability of the test to identify correctly those who do not have the disease. **Positive predictive value** is defined as the proportion of patients who test positive and actually have the disease.

Negative predictive value is defined as the proportion of patients who do not test positive and actually do not have the disease. (9, 10) All these values are calculated using the following formulae:

Sensitivity =	$\frac{\text{True positive (TP)}}{\text{TP} + \text{false negative (FN)}} \times 100$
Specificity =	$\frac{\text{True negative (TN)}}{\text{TN} + \text{false positive(FP)}} \times 100$
Positive predictive value =	$\frac{\text{TP}}{\text{TP} + \text{FP}} \times 100$
Negative predictive value =	$\frac{\text{TN}}{\text{TN} + \text{FN}} \times 100$

Results

A total of 100 blood samples were tested for malarial parasites by light microscopy. The same samples were tested by the ICT malaria Plasmodium falciparum/Pan test device (P.f/Pan). The results of light microscopy and ICT were compared.

Results of Microscopy of the blood films indicated that 60 (60%) patients were infected with malaria; and the rest 40 (40 %) were malaria negative. Among the positive patients *P. falciparum* was detected in 52 (87%) and non-falciparum Plasmodium species were found in the remaining 8 (13%). Mixed infection was found in none of them.

The ICT malaria P.f./Pan Test results showed that 54 (54%) of the patient samples were positive for malaria parasites and the rest 46 (46%) were negative for malaria parasites. Infection with *P. falciparum* accounted for 51 (94.4%) cases of the 54 positives; and non-falciparum Plasmodium species for the remaining 3 (5.5%). Mixed infections were not found. (Table1).

Table 1: Comparison between the results of blood film microscopy and immuno-chromatographic test (ICT) for detection of malaria in Amana clinic, 2020

Test	Result	
	Positive	**Negative**
Blood film microscopy (standard) n =100	60(60%)	40(40%)
ICT for malaria (test) n =100	54(54%)	46(46%)

The results of true positive (TP), true negative (TN), false positive (FP), false negative (FN) will obtained according to (table 2).

The results showed sensitivity of ICT was (89%), and the specificity was (86%) (Table 3), the positive predictive value was (99%) and the negative predictive value was (31.7%) (Figure1), and the validity of ICT was (89.2%). MCC of true positive, false positive, true negative and false negative was 0.78, which was significant with the gold standard method.

Table 2: comparison of results of Malaria ICT and Microscopy.

Test		Microscopy	
		Positive	Negative
ICT (Malaria RDT)	Positive	TP (Positive by both)	FP (Negative by microscopy but positive by ICT)
	Negative	FN (Positive by microscopy but negative by ICT)	TN (Negative by both)

Table 3: Sensitivity and Specificity of ICT for malaria in Amana clinic patients, 2020

Sensitivity and specificity of ICT for Malaria			
Sensitivity =	$\frac{(TP)}{(TP)+(FN)}\times 100$	$\frac{114}{114+14}\times 100$	**89%**
Specificity=	$\frac{(TN)}{(TN)+(FP)}\times 100$	$\frac{86}{86+14}\times 100$	**86%**

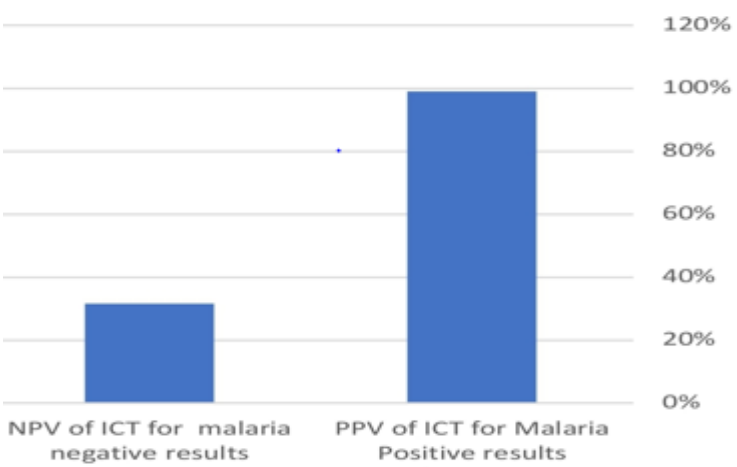


Figure 1: Positive predictive value and Negative predictive value of ICT

Discussion

Although microscopy remains the gold standard method for the diagnosis of malaria, yet there are several constraints such as the lack of trained microscopy technologists, time consumed for microscopy examination and scarcity of good devices. An easily performed, time-effective and accurate test for the detection of Plasmodial infections is needed especially in countries like Sudan with endemic malaria, large population and scarcity of trained laboratory staff. The current study was performed to compare ICT with microscopic malarial parasite detection and to determine the diagnostic validity (sensitivity and specificity) of the ICT method using microscopy as the gold standard diagnostic method. The results showed: the sensitivity of ICT was 89% and the specificity of ICT was 86%; the positive predictive value was 99% and the negative predictive value was 31.7%. These findings are consistent with many similar studies conducted within and outside Sudan. The findings were consistent with a similar study performed in Pakistan that compared the validity of ICT malaria with slide microscopy and showed that the sensitivity of ICT for malaria was 96.1% and specificity was 95.7%. (9) However our results are not in agreement with another study from Pakistan, where sensitivity and specificity results to evaluate the "Optimal Test" for the rapid diagnosis of Malaria in children in 2005 showed a sensitivity of 85% and specificity of 91%; positive predictive value of ICT was 68% and negative predictive value of ICT was 96%. (11) The disagreement may be due to the fact that the reagents of the two studies were directed to two different components of the test. In our study, the ICT was used to detect malaria surface antigens, while the "Optimal Test" was used to detect malaria enzymes. Our results are consistent with the findings of a similar study from Guinea, in which the sample size was 400, the sensitivity of ICT was 93% and the specificity was 95%. (12) Thus, the specificity was higher than our findings. Our findings were consistent with a study performed in Egypt to study the validity of rapid malaria test and microscopy in detecting malaria in a pre-elimination region in Egypt.

The sample size was 600 and the ICT sensitivity was 95%. (13) Another Egyptian study conducted in Mansoorah Hospital, using a sample size of 211 to evaluate sensitivity and specificity of rapid malaria test in reference to light microscopy, reported sensitivity of 98% and specificity of 96%. (14) These results were higher than those shown in our study; the discrepancy may be due to differences in burden of malaria disease between Sudanese and Egyptian population, the malaria burden being higher in Sudan than Egypt. Moreover, there is a likelihood of the Sudanese population having a more effective immune response against malaria. Unlike the above quoted studies, a study from Uganda showed that persistent antigenicity in patient's blood reduces the accuracy and showed low specificity of ICT and other RDTs (15), another study from Equatorial Guinea showed a low ICT sensitivity of 69.7% and a specificity of 73.7%. (16), in Pakistan study of ICT showed 97% Sensitivity and 98.3% Specificity for P.falciprum, and 89.7% sensitivity, and 97.9% Specificity for P. vivax, they concluded that RDTs shouldn't be used without reference method. (17)

Our study and most of the above quoted studies indicate that the sensitivity and specificity of ICT were suboptimal compared to the gold standard method of light microscopy in expert hands. This may be due to some biological factors, such as; the level of malaria parasitemia and cross reactivity with other microorganisms and antigens. Environmental factors may be operating such as: variability of temperature, humidity, transportation factors and storage environment of reagents. (18, 19, 20)

Conclusion:

Although the study shows that the diagnostic accuracy of ICT was suboptimal compared to the gold standard method of microscopy, yet RDTs are valid as a supporting diagnostic tool for malaria. In difficult cases where ICT was used to diagnose malaria, there may be need to use light microscopy in experts hand for the final verdict.

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Authors own resources

Conflict of interest

None

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