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THE USE OF IMMUNOCHROMATOGRAPHIC TECHNIQUE (ICT) IN THE DIAGNOSIS OF MALARIA IN ILORIN, NIGERIA

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Malaria is a major global health problem with about 2.4 billion people at risk. It is the commonest cause of outpatient consultations and one of the leading causes of paediatrics medical admission. Prompt and accurate diagnosis is the key to effective disease management and one of the main interventions of the global malaria strategy. We assess the sensitivity, specificity, predictive values and diagnostic accuracy of immunochromatographic technique (ICT) with the aim of assessing its relevance to the diagnosis of malaria in the North Central part of Nigeria. The study population, which comprised of 39 subjects aged 1 to 49 years, was sent to the hospital laboratory after clinical assessment. Thirty-five (89.7%) of the 39 subjects with fever had parasite count by thick blood film (TBF) ranging from 60-7,200 parasites/μL of blood. Twenty-five of these were positive by the dipstick technique giving a sensitivity, specificity, positive predictive value, negative predictive value and accuracy for the ICT of 71.4%, 100%, 100%, 28.5% and 74.3% respectively when compared with the TBF. All four (4) subjects that were negative microscopically also tested negative with the ICT kit. We therefore conclude that ICT kits is a good alternative in diagnosis of malaria especially in adult in an endemic environment, because it is fast, requires simple manpower and no need of heavy equipment, However, before antigen tests can replace the thick and thin film, it should cover and differentiate between all Plasmodia species and detect lower level of parasitaemia.

INTRODUCTION

Malaria is a major global health problem with about 2.4 billion people at risk. It is estimated world wide that 200-300 million cases occur annually with about 1 million deaths, 90% of which occur in Sub-Saharan Africa (1). Malaria accounts for 10% of Africa's disease burden (2). It is the commonest cause of outpatient consultations and one of the leading causes of paediatrics medical admission (3, 4, 5). Falciparium malaria is the commonest cause of malaria in the Sub-Saharan region, while species other than *Plasmodium falciparium* account for less than 5% of infection (6).

It is estimated that up to 44% of fevers are attributable to malaria (7, 8). Therefore excluding malaria infection as a cause of ill health is central to improved health in this sub-region since other infectious diseases that cause high morbidity and mortality can be treated early following exclusion of a more common disease like malaria (9).

Prompt and accurate diagnosis is the key to effective disease management and one of the main interventions of the global malaria control strategy (10). Clinical diagnosis of malaria is inexpensive to perform and requires no special equipment or supplies. The symptoms of malaria are however non-specific and overlap with those of other febrile illnesses. A diagnosis of malaria based on clinical ground alone is therefore unreliable and when possible should be confirmed by laboratory tests. However, in endemic areas with considerable levels of acquired immunity, asymptomatic infection is common especially in the adult population (11). As high parasite count are more likely to coincide with fever, an alternate approach is to diagnose clinical malaria for fever episode when the parasite count is above a defined value which may

vary according to the level of acquired immunity (12, 13).

Accurate diagnosis of malaria is central to effective disease management and the goal standard is microscopic examination of stained blood film of malaria parasite. However, microscopy is labour intensive and can be inappropriate for some settings due to logistical burden such as lack of trained staff, time and technical equipment. Immunochromatographic test (one step malaria card test) is a rapid test that qualitatively detects the presence of *Plasmodium falciparum* Histidine-rich Protein 2 (HRP-2) antigen in whole blood using two antibodies, one attached to a visible colloidal gold in the test strip and the other immobilized to a membrane strip. It is an easy to use and rapid diagnostic test that requires simple manpower and no equipment support.

Our objective is to determine the sensitivity, specificity, predictive values and diagnostic accuracy of immunochromatographic technique (ICT) with the aim of assessing its relevance to the diagnosis of malaria in the North Central part of Nigeria.

PATIENTS AND METHOD

The study population comprised 39 subjects aged 1 to 49 years who had clinical malaria presenting at the outpatient department of a leading private hospital (Malaria Research Centre, Olanrewaju Hospital) in Ilorin located in the North Central region of Nigeria. The study was conducted between August and October 2002.

Clinical malaria was diagnosed as symptoms of fever, headache, body pains, loss of appetite, nausea and vomiting, abdominal discomfort and dizziness. Those who have commenced therapy elsewhere for malaria were excluded. The controls were patients who had other complaints not related to malaria on presentation.

The subjects were sent to the hospital laboratory after clinical assessment for full blood count, thin and thick blood films. Two milliliters of venous blood were collected from each subject for FBC and blood films. Full blood count was done using Leishmans stain on smeared slide. The thin blood film was used for speciation of the malaria parasite and was read on the same slide used for the FBC. The thick blood films were stained with Geimsa stain. All readings on the light microscope were done using high power 100 X oil immersion objective.

Malaria parasites were counted until 200 white blood cells (WBC) were encountered or up to 500 WBC when the number of parasite per 200 WBC was less than 10. Subjects were considered negative for malaria parasite if 500 WBC were counted without a parasite. The number of malaria parasite per ml was calculated using the formula; Number of parasite encountered (P) X (WBC count of patient)/Number of WBC counted (14). The WBC count used in this study was the average WBC for all the patients under investigation. The 'plus system' was also employed on the thick blood film examined with oil immersion objective and the results recorded using one to four pluses; + = 1-10 parasites per 100 thick film fields, ++ = 11-100 parasites per 100 thick film fields, +++ = 1-10 parasites per thick film fields, ++++ = More than 10 parasites per thick film fields.

Subjects positive for malaria parasite by the blood film were subsequently

tested with the rapid ICT diagnostic kit (Acon laboratories, USA). Other patients negative on blood films but with clinical features of malaria were also tested with the ICT kit. The ICT was performed according to the manufacturer's instruction. Briefly, a disposable specimen dropper was used to pick one drop (about 10 µL) of whole blood which was freshly obtained by finger prick from each subject and transferred to the specimen well of the kit. Three drops of buffer (about 120 µL) were added and the timer started. Results were read after 15 minutes. A pink line at the test and control region of the strip indicate positive result, a pink line at the control lane alone indicate negative result while absence of any pink line or pink line at the test alone indicate an invalid result.

Data were entered into EPI-INFO version 6.0 computers for analysis. Test of significance between variables was ascertained using Chi square test. The main limitation of the study was the cost of the kit.

RESULTS

Thirty five (89.7%) of the 39 subjects with fever, had parasite count by thick blood film (TBF) ranging from 60-7,200 parasites/µL of blood. Twenty-five of these were positive by the ICT dipstick technique giving sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy for the ICT of 71.4%, 100%, 100%, 28.5% and 74.3% respectively when compared with the TBF as "gold standard". All four (4) subjects who were negative microscopically also tested negative with the ICT kit. (Table1).

Table 2 shows a comparison of 2 malaria parasite counting method; parasite count per liter and the "plus" method commonly used in this environment. Seven of the 10 patients with negative ICT had parasite count below 100/µL while 2 had parasitaemia level between 100 and 300/µL. When the 7 patients were removed from the analysis, the sensitivity and negative predictive values increased to 92.6% and 100% respectively. The test was negative at parasite count below 300/µL showing 0% sensitivity. There were no false positives and no invalid results. The WBC used in calculating the parasite count was 6,000/µL (6 X 10^9/L), which is the average WBC count recorded among the patients.

Table 1: Laboratory result of thick blood film (TBF) and immunochromatographic technique (ICT)

Diagnostic method		TBF		Total
		Positive	Negative	
ICT	Positive	25	0	25
	Negative	10	4	14
Total		35	4	39

Table 2: Comparison of two malaria parasites counting methods; Parasite count per microliter and the "Plus" method

Parasite count / µL	"Plus" system	No positive (TBF)	No positive (ICT)
> 4,500	++++	2	2
1,800-4,500	+++	8	8
900-1800	++	4	4
300- 900	+	12	11
100-300	scanty	2	-
< 100		7	-
Total		35	25

DISCUSSION

The average WBC among the patients recruited into this study is 6.0 x 10^9/L. This is in contrast with findings in caucasians in which an average of 8.0 x 10^9/L has been generally accepted (14). This calls for reassessment of average values in health and disease among people in this environment. The finding of 100% *Plasmodium falciparium* among the patients in this study agrees with other reports in which over 95% of plasmodia causing malaria is *Plasmodium falciparium* (5, 15, 16). However, this contrasts the findings of Wolday *et al* (17) in Ethiopia in which 32.8% were *Plasmodium falciparium* and 66.4% were *Plasmodium vivax.*

In this study, the dipstick method was persistently positive at a parasite count above 300/μL. This is less sensitive than the work done earlier by Olanrewaju *et al* (5) in which the kit was persistently positive at a count above 100/μL. Though, only 71.4% sensitive when compared with the thick film, the sensitivity of this kit approach 100% at a parasite count above 300/μL. The sensitivity (71.4%), PPV (100%) and NPV (28.5%) are comparable with the findings of Olanrewaju *et al* (sensitivity 66.7% and PPV 100%) and Wolday *et al* (sensitivity 97.2%, PPV 77.8%. Similar to findings of Olanrewaju *et al,* there was no invalid result and no doubtful case unlike that of Proux *et al* (18). The larger number of subjects rather than kits used by Proux *et al* can be said to account for this difference.

There remained an unresolved issue that for a patient in malaria endemic area where asymptomatic infection is commonly seen (11), what level of parasitaemia will be clinically relevant to the diagnosis of malaria (15). Further and more elaborate studies will help in providing information on the level of parasitaemia at which malaria infection can be conveniently diagnosed. Possible effort at having multiple lines on the kit that can determine an increasing plasmodium parasite/μL of blood (graduated kit) on the part of the manufacturers of this product will be relevant to enhance the usefulness of the kit. Moreover, ICT kit will be more relevant to malaria diagnosis if the parasite count at which the kit becomes positive can be noted and reflected in each batch by the manufacturer.

We therefore conclude that ICT kit is a good alternative in the diagnosis of malaria especially in adult in an endemic environment, because it is fast, requires simple manpower and no need of heavy equipment. There is however the need for us to determine the level of parasitaemia corresponding to malaria disease in this area to reduce the problem of over-diagnosis of malaria. Finally, before antigen tests can replace the thick and thin film, it should differentiate between other plasmodia species and detect lower level of parasitaemia.

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