Evaluation of Malaria Parasite Screening Procedures Among Sudanese Blood Donors

MOHAMED SIDDIG M ALI, ABDUL GADER MOHAMED YOUSIF, MUSTAFA SALIH MUSTAFA, MALIK HASSAN IBRAHIM

OBJECTIVE: To compare the standard microscopic examination, the polymerase chain reaction (PCR), and the immunochromatography test (ICT) to determine the best method for screening blood donors for malaria parasites in Sudan.

METHODS: A total of 100 blood donors were screened for malaria parasites by standard microscopic technique, ICT, and PCR. Blood films were examined microscopically using standard Giemsa staining techniques. Qurum (Canadian Company) malaria kits were used to perform the ICT. For performing PCR, DNA was extracted using Chelex method and amplified by the moderately repetitive DNA sequence pBRK-l.

RESULTS: Using PCR, a total of 21 blood samples were positive; 8 (38%) of them showed negative blood films and 7 (33%) were negative on ICT. Four blood samples that tested positive by ICT despite a negative PCR and microscopic examination were proved to be false positives. The false negativity of both the microscopic examination and ICT was found to be significant. The sensitivity of microscopy was 61.9% and of ICT was 66.7%, while the specificity of microscopy was 100% and of ICT was 94.9%. When direct microscopy was considered as the standard technique the sensitivity of ICT was 100% and the specificity was 94.3%.

CONCLUSION: Although PCR is more sensitive and more specific, it is unaffordable. Microscopy for malaria when compared to ICT showed similar sensitivity at low cost. However, all human plasmodium species can be detected using the microscopy while only two species (*P. falciparum* and *P. vivax*) can be detected by ICT. The detected false positivity of ICT is not inconsequential since this implies the rejection of a greater proportion of blood donations. Therefore, microscopy is considered more suitable for screening Sudanese blood donors for malaria parasites prior to donation at the present time.

RECOMMENDATIONS: To establish a reference malaria diagnosis unit in each blood bank in Sudan as well as to train blood bankers to perform microscopic examinations.

ABBREVIATIONS: ICT = immunochromatography test; PCR = polymerase chain reaction.

INDEX TERMS: donor testing; malaria testing.

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Dr Mohamed Siddig M Ali MSc Haematology *is Lecturer and Head, Department of Haematology, AI Neelain University, Khartoum, Sudan.*

Abdul Gader Mohamed Yousif PhD *is Professor of Medicine and Tropical Diseases and Dean, Faculty of Medicine, University of Khartoum, Khartoum, Sudan.*

Mustafa Salih Mustafa MD *is General Manager of Planning and Development and Research Directorate, Federal Ministry of Health, Khartoum, Sudan.*

Dr Malik Hassan Ibrahim MSc Haematology *is Lecturer and Head, Department of Haematology, Sudan University of Science and Technology, Khartoum, Sudan.*

Address for correspondence: *Dr Mohamed Siddig Mohamed Ali, Faculty of Medical Laboratory Sciences - AI Neelain University, PO Box 12702, Khartoum, Sudan. mohdaru@hotmail.com*

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The medical use of blood and blood derivatives is increasing all over the world, despite the hazards related to transmission of protozoal, spirochaetal, bacterial, and viral diseases. Transmission of malaria by blood transfusion is a significant problem in regions of the world where this disease is endemic; *P. falciparum* in transfused blood may lead to fatality.[1] Moreover, transfusion-induced *P. falciparum* has increased in recent years, probably because it has become increasingly resistant to drugs.[2]

Prevention of transfusion-induced malaria depends on the screening of potentially infected blood donors, especially those who provide whole blood or fresh concentrates of erythrocytes, leukocytes, or platelets.[3] However, successful screening for malaria parasites requires experience in the differential characteristics of the various species of the parasite and should constitute a part of primary health care.[2] Microscopic examination of a blood film can be used for the detection of malaria infection in a donor in whom malaria is suspected based on circumstantial evidence. Examination of stained thin blood films can identify the species and give an estimate of the percentage of infected red cells.[4-6] Examination of the buffy coat and red cells below can help in detecting parasites when they are few in number (5 to 10 parasites/μL), particularly in the late stages, e.g., trophozoites and gametocytes.[5] However, in traditional microscopy, failure to adhere to correct techniques seriously compromises the specificity and sensitivity.

Immunochromatography tests (ICT) employ monoclonal antibodies against histidine rich protein (HRP-2), which is produced by the parasite and released into the circulation. Compared to microscopy, it is simple to perform, does not require the use of special equipment, and is faster with low variation between users. Moreover, it can detect asexual parasites and young gametocytes with reasonable sensitivity and specificity (>90%).

Serological methods cannot replace the demonstration of parasites in the blood as far as diagnosis of symptomatic patients is concerned.[6] In countries where the disease occurs, antibody tests are unable to distinguish between active and past infection and they have only a limited value in the clinical diagnosis of malaria.[5]

During the past few years, the polymerase chain reaction (PCR) has become a major diagnostic and research technique. It is reliable for the detection of parasites present at low concentration in blood or serum samples. Further, the use of PCR could be 100 times more sensitive than the microscopic examination of thick blood films.[7]

The present study is intended to determine the selection of the best laboratory procedure that can be applied for screening blood donors for malaria parasites in Sudan since such studies have not been conducted previously.

MATERIALS AND METHODS

The design of the study is descriptive, cross-sectional, and facility based. It was conducted in the Ahmed Gasim Hospital (Khartoum North, Sudan) among a total number of 100 blood donors screened for malaria parasites by ICT, PCR, and standard microscopic technique. Blood samples were collected (1 mL from each), processed with EDTA anticoagulant (1.5 mg), and then immediately used to prepare blood films and perform ICT. Three spots of each tested blood sample (50 μL each) to be used for the PCR were stored dry (on #3 filter paper) at -20 °C.[8]

From the blood collected from each donor, duplicate thick and thin blood films were prepared, stained by Giemsa stain, and examined microscopically as the standard methods.[5]

The absolute number of parasites (number/μL), was estimated in positive thick blood films by counting the recognized malaria parasites against 200 white blood cells according to the following formula:

$$\frac{\#\text{ of parasites counted} \times \text{total WBC}}{\#\text{ of leukocytes counted (200)}}$$

Qurum (Canadian Company) ICT malaria kit was used. This kit has been designed for the detection of *P. falciparum* in whole blood. It employs monoclonal antibodies against HRP-2 that is secreted by the parasite. Captured monoclonal antibodies are immobilized on a nitrocellulose membrane strip. When *P. falciparum* antigen is present in lysed whole blood, it binds antibodies as the blood migrates along the test strip. Colloidal gold particles are coated with these antibodies to form a sandwich resulting in visible red line. Performance of the test and interpretation of results were conducted as directed by manufacturer instructions.

For performing PCR, DNA was extracted from the filter paper using Chelex method.[8] One of the three blood spots was cut using a sterile blade and put in a 1.5 mL Eppendorf tube containing 1 mL of 0.5% saponine in freshly prepared 1 x phosphate buffer saline (PBS) and then incubated overnight at 4 °C (hemoglobin is released into the wash leaving the DNA of the parasite on the paper).

The tubes were spun for one minute at 13 x 10^3 rpm and the supernatant fluid was removed. One mL of 1x PBS was added and spun again for one minute; the supernatant was also removed. Fifty μL of PCR quality water and 50 μL of 20% Chelex mixture were added to each tube and then boiled for ten minutes.

The tubes were centrifuged for one minute to pellet Chelex and debris. The DNA supernatant was taken off and transferred into a new sterile 0.5 mL Eppendorf tube.

DNA amplification was performed using the moderately repetitive DNA sequence pBRKl- 14 forward, 5-CGC TACATATGCTAG TTGCCA GA C-2′ and reverse 5′-CGTGTA CCATA CATCCTACCAAC-3′ that amplifies a 206 base pair sequence.[7] The PCR product was run in a gel electrophoresis tank (1.5% agarose gel) with the addition of ethidium bromide solution (0.5 mg/mL) and DNA molecular weight marker (fraction VI) in a parallel well.

Data were analyzed by the computer using the Statistical Package for Social Sciences (SPSS). Chi-square and Fisher exact tests were used for comparison and correlation between proportions.

The sensitivity of the test is the proportion of positives, corresponding to the positive result obtained by the standard test. However, specificity of the test is the proportion of negatives corresponding to the negative result obtained by the standard technique. Sensitivity, specificity, false negative rate, and false positive rate can be calculated using the following formulas and Table 1:

Sensitivity = (e)/(e + f)
Specificity = (h)/(g + h)
False negative rate = 1 – sensitivity
False positive rate = 1 – specificity

RESULTS

Out of the 100 randomly selected blood donors, 13 samples (13%) were positive by microscopy as well as by ICT and PCR, with 75 samples (75%) being negative throughout. Twelve blood samples (12%) showed variable results.

Table 1. Sensitivity and specificity table

Tested technique	Standard technique		
	Positive (D+)	Negative (D-)	Total
Positive (T+)	(e)	(g)	(e + g)
Negative (T-)	(f)	(h)	(f + h)
Total	(e + f)	(g + h)	

Comparison between the results of PCR and ICT of the examined blood samples applying PCR as the standard technique is shown in Table 2. Using PCR, a total of 21 blood samples were positive; of them only 14 blood samples tested positive using ICT. Four blood samples tested positive by ICT despite a negative PCR and microscopic examination; therefore, these proved to be false positives. The false positive of the ICT was found to be statistically insignificant (p >0.05).

Seven (33%) positive PCR blood samples were negative by ICT. These false negative ICT results were found to be highly significant (p <0.001).

Applying PCR as a standard technique, the sensitivity of ICT was 66.7% while the specificity was 94.9%. The false negative rate was 33.3% and the false positive rate was 5.1%. Comparison between the result of PCR and microscopic examination of the donors' blood samples applying PCR as the standard technique is shown in Table 3.

Using PCR as the standard technique, a total of 21 blood samples were positive; 8 (38%) of them showed negative blood films. This false negativity of the microscopic examination was found to be highly significant (p <0.001).

Table 2. Comparison between the results of PCR and ICT

	PCR		Total
	Positive	Negative	
ICT positive	14	4	18
ICT negative	7	75	82
Total	21	79	100

p >0.01

Table 3. Comparison of PCR vs. microscopic examination

	PCR		Total
	Positive	Negative	
Microscopic examination			
Positive	13	0	13
Negative	8	79	87
Total	21	79	100

p >0.01

Applying PCR as a standard technique, the sensitivity of microscopic examination was 61.9% while the specificity was 100%. The false negative rate was 38.1% and the false positive rate was zero. Comparison between the result of microscopic examination and ICT of the same blood samples applying microscopy as the standard technique is shown in Table 4.

Using microscopic examination, a total of 13 blood samples were positive; 5 (38.5%) of them were ICT negative. Only one donor's blood sample (7.7%) was found positive by ICT and PCR despite a negative blood film.

The false positive ICT (the other four positives; 22.2%) which were negative by blood films, representing the difference between microscopy and ICT, were found to be insignificant (p >0.05).

Applying microscopic examination as standard technique, the sensitivity of ICT was 100% and the specificity was 94.3%. The false positive and false negative rate was 5.7% and zero respectively.

DISCUSSION

Transfusion-induced malaria continues its resurgence throughout much of the tropics and subtropics. Successful control or eradication measures must include strategies directed to prevent transmitting malaria parasite infected blood to patients. Therefore, it is mandatory to establish an effective technique for screening blood donors for the malaria parasite. Different techniques were compared to determine the best method of screening donors that can be easily and rapidly applied in Sudan.

Since PCR is highly specific and highly sensitive for positive and negative results respectively (100 times greater than staining technique), it can be used as a standard technique to avoid false negatives and positives.[7] In this study, PCR was adopted as the standard technique. It is worthwhile to mention that during this investigation, the difference between PCR and both ICT and direct microscopy was still significant, although PCR has the drawbacks of being costly and requiring strict laboratory conditions to avoid contamination that can lead to false positive results.

Table 4. Comparison between the result of microscopic examination and ICT of the same blood samples applying microscopy as standard technique

	Microscopic examination		
	Positive	**Negative**	**Total**
ICT positive	13	5	18
ICT negative	0	82	82
Total	13	87	100

p =1.20

In malaria-endemic areas, the use of ICT is known to be rather deceptive when used for investigating symptomatic patients for the presence of malaria parasite in their blood. False positive results can be obtained even 14 days after clearance of the parasites by treatment or by the immune system.[5,6, 9-11]

This study showed that sensitivity of the ICT versus direct microscopy was 100%, which corresponds to the results obtained by Singh in India, Cavallo in France, and Gaye in Senegal.[11-13] However, all of them confirmed the false positivity of ICT within variable periods after infections. These reports are in close agreement with the findings of this study; four false positive tests were detected. The false positive results are important since this implies that a greater proportion of blood donations will be rejected to ensure the prevention of malaria transmission by blood transfusion. Therefore, ICT seems to be not reliable for screening Sudanese blood donors for malaria parasite since whether the parasite is present or not, persistence of HRP-2 will result in false positivity. Further, only ICT designed for the detection of only two species (*P. falciparum* and *P. vivax*) is available, whereas transfusion induced malaria due to the other human plasmodium species (*P. malariae* and *P. ovale*) is not uncommon, particularly *P. malariae* because of its chronicity. The ICT test will be falsely negative when the latter species are present.

The standard microscopic examination technique for malaria parasites enables the detection of all four human plasmodium species. It also allows distinction between species and stages of infestation and the capability of determining parasitaemia which may produce some new epidemiological and parasitological aspects providing some suggestions for eradication; moreover, it is cheap, relatively rapid, readily available, and easy to perform.

When compared with ICT, microscopic examination shows similar sensitivity (no statistical difference; p >0.05). Hence, microscopy of malaria is more suitable for screening blood donors in Sudan. This is in agreement with Wilairat's findings that microscopic diagnosis of malaria is still more than adequate

in the routine investigation of individual cases, in spite of the fact that many authors have not favored the use of this method because of the amount of time and expertise required.[14]

There is still no international consensus for the exact definitions of what constitutes high, intermediate, or low parasitaemia; undoubtedly, most of the parasite densities that were encountered in our study were relatively low. However, the minimum number of parasites transmitting malaria via blood transfusion may vary among individual recipients; a single parasite can transmit the disease in mice.[13] Even if a single parasite can be detected in a thick blood film, which is equivalent to 4 μL of blood, more than hundreds of thousands of parasites might escape in a full unit of blood (450 mL).

This signifies the importance of the reliable testing of blood donors for malaria parasite to minimize, though never completely eliminate, the risk of malaria transmission by blood transfusion. Moreover, the hazards of transfusion malaria are serious, and justify prior testing of blood donors for malaria even by advanced expensive techniques. These techniques will surely reduce the false negative results and hence minimize the risk of transfusing infected blood.

The standard microscopic identification technique of malaria parasites is ideal to be applied at the present time until the development of more feasible application of PCR. The establishment of a reference malaria diagnosis unit in each blood bank as well as trained blood bankers is necessary.

CONCLUSION

The hazards of transmitting malaria through blood transfusion are serious thus justifying the use of expensive techniques for testing blood donors. Transfusion of blood from malaria parasite infected donors to patients will result in transfusion-malaria; screening donors for malaria prior to donation will undoubtedly reduce this risk. Microscopy is much cheaper than both ICT and PCR; in addition, it enables the detection of all human plasmodium species. Therefore, it is the best technique to be adopted for the control of transfusion-induced malaria in the different regions of Sudan until the feasibility of using PCR technology is improved.

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REFERENCES

1. Barker RH, Banchongaksorn T, Courval JM, and others. A simple method to detect *Plasmodium falciparum* directly from blood samples using the polymerase chain reaction. Am J Trop Med Hyg 1992;46:416-26.
2. Neva FA, Brown HW. Basic clinical parasitology. 6th ed. New York: Appleton and Lange; 1994. p 347.
3. Bruce Chwatt LJ. Transfusion malaria revisited. Trop Dis Bull 1982;79(10):827-40.
4. Sherman IW. Malaria. Washington: ASM: 1998. p 557.
5. Cheesbrough M. Medical laboratory manual for tropical countries. 2nd ed. Oxford: Butterworth; 1987. p 602.
6. Manson Bahr PEC, Bell DR. Mansons' tropical diseases. 19th ed. London: ELBS; 1987.
7. Vu TT, Tran VB, Phan NT, and others. Screening donor blood for malaria by polymerase chain reaction. Trans R Soc Trop Med Hyg 1995;89:44-7.
8. Tirasophon W, Panyim S. PCR for low level detection of malaria parasite in blood. Protocols in molecular parasitology In: Hyde JE, editor. Methods in molecular biology. 21st ed. Totowa NJ: Humana Press; 1993. p 205-12.
9. Karbwang J, Tasanor O, Kanda T, Wattanagoon Y. ParaSight ™-F test for the detection of treatment failure in multi-drug resistant *Plasmodium falciparum*. Trans R Soc Trop Med Hyg 1996;90:513-5.
10. Valecha N, Sharma VP, Devi CU. A rapid immunochromatographic test (ICT) for diagnosis of *Plasmodium falciparum*. Diagn-Microbiol Infect Dis 1998;30(4):257-60.
11. Singh B, Cox-Singh J, Miller AO, and others. Detection of malaria in Malaysia by nested polymerase chain reaction amplification of dried blood spots on filter papers. Trans R Soc Trop Med Hyg 1996;90(5):519-21.
12. Cavallo JD, Hernandez E, Gerome P, and others. Serum HRP-2 antigens and imported *Plasmodium falciparum* malaria. Comparison of ParaSight-F and ICT malaria P.f. Med Trop 1997;57(4):353-6.
13. Gaye O, Diouf M, Dansokho EF, and others. Diagnosis of *Plasmodium falciparum* malaria using ParaSight F and ICT malaria P.F. Parasite 1998;5(2):189-92.
14. Wilairat P. DNA probes for malaria parasite detection: current status and future prospects. In: Ko RC, editor. Immunological and molecular basis of pathogenesis in parasitic diseases. Hong Kong: Hong Kong University Press; 1989. p 169-74.