

Transfusion-transmitted Malaria in Khartoum, Sudan: Comparison of Microscopy, Rapid Diagnostic Test and Nested PCR Methods

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Abstract

Malaria can be efficiently transmitted by the transfusion of cellular blood components. It is undoubtedly responsible for the majority of transfusion transmitted diseases in the world. The World Health Organisation recommends that donated blood should be tested for malaria “where appropriate and possible”, but there is currently no method for screening blood for low-level parasitaemia that is sensitive, practical and affordable for use by transfusion services in endemic countries. This study aimed to determine the prevalence of malaria parasitaemia in blood transfusion donors in Khartoum, Sudan; and to evaluate three diagnostic tests: microscopy, rapid diagnostic test and molecular technique, for the detection of malaria parasites. Following informed consent Two hundred blood donors were enrolled. Two ml of EDTA anticoagulated blood was collected from each donor for Conventional thin and thick blood smears, an immunochromatographic test, and molecular technique. Malaria parasites were detected in 6% by microscopic examination, 6.5 % by immunochromatographic test and 18.5 by molecular technique. In conclusion, we determine high prevalence of malaria parasitaemia among blood transfusion donors in Khartoum state, Sudan, with variable detection sensitivity between the three different techniques. Our finding

highlighted the urgent need to develop an effective strategy for the prevention of transfusion-transmitted malaria in Sudan.

Keywords: Malaria, Blood transfusion, Sudan.

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Introduction

Malaria is one of the most important parasitic diseases in the world and remains a major challenge to mankind. The disease occurs mostly in tropical and subtropical regions, particularly in sub-Saharan Africa and Southeast Asia (1). About 90% of all malaria infections in the world occur in Africa South of the Sahara. Majority of infections in the region are caused by *Plasmodium falciparum*, the most dangerous and the most effective malaria vector of the four human malaria parasites (2).

The administration of blood to a patient is potentially a lifesaving procedure and the demand for blood has greatly increased over the years (3). Although this therapy helps to save lives, blood can nonetheless be a vehicle for transmission of infections including parasitic diseases (4). Malaria can be efficiently transmitted by transfusion of cellular blood components, and it is undoubtedly responsible for the majority of transfusion transmitted diseases in the world (5,6).

Induced malaria by blood transfusion was first reported in 1911 (7,8). It is well established that all four human malaria parasites (*P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*) may be transfusion-transmitted (9). The fact that, malarial parasites can survive in red blood cells at refrigerator temperatures (2-6°C) for days or weeks (10).

When malaria is transmitted through blood transfusion to a non-immunized recipient, it can progress rapidly and may lead to significant morbidity and mortality, specifically when diagnosis is delayed (6,11). There are no evidence-based international guidelines for the prevention of transfusion-transmitted malaria (TTM) in sub-Saharan Africa, and there is lack of harmonisation between policies produced by blood safety programmes and policies by malaria programmes (6,12). The World Health Organisation (WHO) recommends that donated blood should be tested for malaria “where appropriate and possible” (13), but there is currently no method for screening blood for low-level parasitaemia that is sensitive, practical and affordable for use by transfusion services in endemic countries (14). This study aimed to determine the prevalence of malaria parasitaemia in blood transfusion donors in Khartoum, Sudan; and to evaluate three diagnostic tests: microscopic detection, rapid diagnostic test (RDT) and molecular technique, for the detection of malaria parasites.

Materials and Methods

Following informed consent Two hundred blood donors , who were attended the central blood bank in Khartoum state – Sudan, were enrolled in this study. Two ml of EDTA anticoagulated blood was collected from each donor for Conventional thin and thick blood smears, an immunochromatographic test (ICT), and molecular analysis. Microscopic examination and ICT were performed at the department of haematology, faculty of medical

laboratory sciences, Alneelain University, Sudan. Molecular detection of malaria parasite was performed at the institute of endemic diseases, Khartoum, Sudan.

Conventional thin and thick blood smears were made and stained with 10% Giemsa (at pH 7.2). The slides were subsequently examined for the presence of malaria parasites under oil immersion ($\times 100$ magnification). Samples with no visible parasites after scoring 100 fields were considered to be negative for this test. Malaria HRP2 One Step RDT (ABON Biopharm, China) was performed for the detection of plasmodium falciparum according to manufacturer's instructions, in brief, whole blood was added into sample wells and an assay buffer were added into assay buffer wells. The blood -buffer mixture were allowed to run toward the test and control window.

For molecular detection of malaria parasite, DNA was extracted by Guanidine chloride method. Blood samples were subjected to digestion by adding 10 μ L of 20mg/ μ L proteinase K, 300 μ L of 7.5 M Ammonium Acetate and 1 mL of 6M Guanidine chloride then incubated at 37°C overnight, samples were cooled down at room temperature and transferred into falcon tubes containing pre-chilled Chloroform and centrifuged. The supernatant was collected and 10 ml of prechilled absolute Ethanol was added and mixed gently. Samples were incubated at -20°C for overnight. Washing with 5mL 70% Ethanol was performed. Finally the DNA pellet was re-suspended in 200 μ L of deionized water and was incubated at 4°C for overnight allowing the DNA to fully dissolve. DNA purity and quantity was assessed using Nanodrop 100 spectrophotometer. DNA was stored at -20°C.

A Genus and species PCR using primers that target the small subunit rDNA (ssrDNA) specific for the four human malaria parasite species were used as previously described (15). Primer used for genus specific (outer primers) were rPLU5 5' CCTGTTGTTGCCTTAAACTTC 3' , and rPLU6 5' TTAAAATTGTTGCAGTTAAAACG 3' ; and nest primers for species-specific were Fal 1 5'

TTAAACTGGTTTGGGAAAACCAAATATATT 3', and FAL 2: 5'-ACA CAA TGA ACT CAA TCA TGA CTA CCC GTC-3'; to amplify the *P. falciparum* rDNA . In brief, The extracted DNA (2 µl) was used as a template in 25 ml PCR reactions, which contained 0.2 mM of each oligonucleotide primer (Rplu5 and RPlu6 primers), 1× PCR buffer (Vivantis,, Malaysia), 2.0 mM MgCl₂, 0.12 mM dNTPs and 0.03 U/ml Taq DNA polymerase. The PCR was started with an initial step of 5 min at 94 °C, followed by 40 cycles including incubation for 30 s at 94 °C, 1 min at 55 °C and 90 s at 72 °C. The reaction mixture was finally heated at 72°C for 10 min. The amplified PCR products were analyzed immediately by electrophoresis on 2% molecular grade agarose gel (Caisson, USA) and visualized by UV trans-illumination (BioDoc-It UVP, Cambridge, UK) after Ethidium bromide staining. The number and size of DNA fragments was estimated using 100 bp DNA ladder (Vivantis,, Malaysia).

Results

The study included 200 blood transfusion donors. All donors were male; their median age was 32 years with minimum age of 18 and maximum age of 50 years. All blood samples were tested for the presence of malaria parasites by thin blood film, ICT and PCR. As indicated in Table 1, malaria parasites were detected in 6% by microscopic examination, 6.5 % by ICT and 18.5 by molecular technique.

Table 1 frequency of malaria parasitaemia among donors by the three diagnostic methods

	Plasmodium falciparum frequency (%)	Other plasmodium species frequency (%)	Total frequency (%)
Blood film	11 (5.5)	1 (0.5) (Vivax)	12 (6.0)
ICT	13 (6.5)	Not tested	13 (6.5)
PCR	37 (18.5)	Not tested	37 (18.5)

Discussion

In this study we determined the prevalence of malaria parasitaemia in blood transfusion donors, who did not manifest clinical signs of malaria (no fever or recent history of fever and/or malaria). We also evaluated three diagnostic methods for the detection of malaria parasites, namely microscopic detection, ICT and molecular technique. Malaria parasites were detected in 18.5% of the study group by molecular technique. The study population comprised adult volunteers who, during their life, have acquired some degree of immunity against malaria, at low parasitaemia, these individuals do not feel sick, nor have clinical signs of malaria. The PCR technique can detect parasites below the threshold levels of microscopy (0.004 to 1 parasite/ml of blood) (7,16). However, the result directly depends on the quality of the genetic material (DNA) of the parasite obtained during extraction and amplification, and on the quality of the reagents. Furthermore, the test is very expensive, requires extensive training and a long analysis time (7,17).

In routine practice, the “gold-standard” technique, optical microscopy in thick blood smears, is the most often used for Plasmodium detection in malaria-endemic areas. This technique is considered the most effective and inexpensive for the diagnosis of malaria. But despite their continued application as key diagnostic tests, microscopic techniques have some major limitations that render them inappropriate for universal or targeted donor screening. Precisely, they lack the required sensitivity to detect all infected units, specifically in situations of low parasite density (17). It failed to detect the presence of malaria parasite in more than 60% of positive cases in the study group in which malaria has been detected by PCR. Furthermore it is time-consuming (generally requiring one hour or more for preparation and detailed examination).

The detection of malarial antigen with RDTs was originally intended as a more rapid and objective alternative to direct microscopy (7). RDTs detect Plasmodium-Specific parasite

proteins, such as pan-malarial lactate dehydrogenase (pLDH), and *P. falciparum* specific histidine-rich protein 2 (HRP2). Most of these assays are in a 'dipstick' format that can be used with minimal training, are field applicable, and provide a result within 10-20 minutes (7,18). However, RDT method, in our study, has not offered improved sensitivity over microscopy. It detects the presence of malaria parasites in 6.5% of the study group.

Overall, a number of factors need to be considered in selecting the most appropriate method of detection. In general, a balance has to be found between screening needs and the resources available, including finances, staff and their level of expertise, equipment, consumables, and disposables.

Conclusion

In conclusion, we determine high prevalence of malaria parasitaemia among blood transfusion donors in Khartoum state, Sudan, with variable detection sensitivity between microscopic examination, ICT and molecular technique. Our finding highlighted the urgent need to develop an effective strategy for the prevention of transfusion-transmitted malaria.

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