Evaluating laboratory screening tests for malaria on blood donor candidates to reduce the risk of transfusion-transmitted malaria in an endemic area of Indonesia

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ABSTRACT

BACKGROUND Laboratory screening of blood donors for malaria has not been routinely performed in Indonesia. Current policy and practice simply exclude donors based on a history of active clinical malaria. This study was aimed to evaluate laboratory screening tests for malaria among blood donors in an endemic area of Indonesia.

METHODS The study was conducted on 550 consecutive blood samples withdrawn from volunteer donors at the Red Cross Blood Transfusion Unit in Ambon city using microscopic and rapid diagnostic tests for antigen as well as for antibody. Furthermore, 248 of those 550 samples were also tested for the presence of malaria DNA using 18S rRNA marker. Statistical analysis was done descriptively using SPSS software version 15 (SPSS Inc., USA).

RESULTS The overall malaria positivity rate among the donors was 4.5% (25/550). None of the specimens tested using microscopy or rapid test for malaria antigen assay were positive. However 22 (4.0%) samples were positive for malaria antibody against *Plasmodium falciparum*, while 3 (1.2%) were positive by PCR.

CONCLUSIONS Laboratory testing for blood donors may be used to prevent transfusion-transmitted malaria in an endemic area of Indonesia.

KEYWORDS blood donors, malaria, Indonesia

Malaria is an infectious parasitic disease transmitted naturally through bites of female *Anopheles* mosquitoes, but it may also be acquired congenitally or by blood transfusion or organ transplantation.[1,2] Transfusion-transmitted malaria (TTM) occurs because plasmodial parasites survive blood banking processes for 14 days of storage at 4°C.[3] Moreover, a previous study demonstrated that TTM might occur even with a parasite density as low as 1–10 parasites/500 ml unit of blood.[4] Currently, no diagnostic tool is available to detect malaria parasites at such a low density.[5] Thus, the possibility of TTM should always be considered in the event of post-transfusion febrile illness in a recipient, especially when donors reside in malaria-endemic areas. TTM is mostly transmitted via blood products, including whole blood, packed red cell, platelets, leukocytes, and plasma.[1,2,4–6] TTM, as with all diagnoses of malaria,

should be treated as a clinical emergency – delayed diagnosis or inappropriate therapy may quickly lead to complicated illness and significant risk of death.[6,7]

The magnitude of TTM as a clinical problem varies widely, depending almost wholly on risk of malaria occurring among the donor pool. The incidence of TTM in developed countries is extremely low (<2 cases per million units of blood transfused). In contrast, where endemic malaria occurs, TTM incidence may reach 50 cases per million units of blood.[5] Different strategies have been implemented for the prevention of TTM. In most developed countries, prevention measures against TTM include excluding blood donation by recent travelers to and ex-residents of malaria-endemic countries. There are various policies on deferral time for some countries, e.g. in Canada and USA, where antibody screen testing is not performed, longer deferral times are applied (i.e., 1–3 years); whereas countries performing antibody screen testing (e.g., Australia, England, and France), the deferral time is only 4–6 months.[8] The threat of TTM does not typically come from travelers, but from migrants who had resided where endemic malaria prevails.

The malaria screening tests typically used in blood banking in developed countries usually detect antibodies, which was assessed by enzyme-linked immunosorbent assay (ELISA)-based methods employing *Plasmodium falciparum* and *P. vivax* antigens.[8] Meanwhile, blood banks in malaria-endemic regions in Africa and Asia seem to employ various tests, including microscopic examination of Giemsa-stained blood smear, rapid test for malaria antigen, or malaria antibodies.[5,9] More typically, however, most low-income endemic countries have a lack of laboratory capacity to carry out malaria screening of any sort. Although Indonesia has over 120 million people living at risk of malaria and has conducted routine screening test of blood donations for syphilis, hepatitis B, hepatitis C, and HIV, the Indonesian Red Cross has not carried out malaria screening test for blood donors.[10–12] Patients with a history of fever in the prior week are not eligible to donate blood. This strategy presumed that natural immunity to malaria among Indonesians to be relatively rare, and those with active malaria would indeed be acutely ill. However, the presumption has been recently challenged by the finding of a dominance of asymptomatic carriers in most endemic settings in Indonesia.[13–15] Thus, the risk of TTM with blood transfusion may be much higher than has been historically perceived. This study aimed to evaluate several malaria diagnostic tests on blood donor candidates to reduce the risk of malaria transfusion in an endemic malaria area of Indonesia.

METHODS

Study site and population

Ambon city lies in the Maluku archipelago in Eastern Indonesia (3°–4° south and 128°–129° east). The annual parasite incidence (API) was 4.49‰ in 2011 and 2012 and in 2013 was 4.14‰ at the time of samples collection (Ambon District Health Office). The Indonesian Red Cross Transfusion Service in Ambon city received blood from an average of 479 donors/month in 2012; whereas the average number of patients receiving transfusion services was 185 patients/month (Ambon Unit Blood Transfusion).

Blood donor screening

Donor candidates who voluntarily visited the transfusion unit from May to July 2013 were examined and selected in this study following the inclusion and exclusion criteria (Figure 1). Measurements of body weight and vital signs (blood pressure, pulse rate, and respiratory rate) as well as hemoglobin (Hb) levels were performed to ensure that potential donors were in good health. Prospective female donors should not be pregnant or in menstrual cycle. Age limitation was between 17–60 years. For laboratory assessment, 3 ml of venous blood were collected directly from the transfusion set of each individual and transferred into EDTA tube for all laboratory testing (hepatitis B & hepatitis C, HIV, syphilis, and later rapid malaria tests for this study). We only analyzed blood from donations considered eligible for transfusion; thus, all of whom were considered healthy and free of febrile illness for at least 1 week.

Microscopic examination of Giemsa-stained blood films

A finger prick by lancet was done for blood films on microscope slides. Thick and thin blood smears were stained with 3% Giemsa solution for 40 min, rinsed and dried, and finally read by a qualified technician using a binocular microscope magnified 1,000x with immersion oil. The slides were considered negative for malaria when no malaria parasite was detected after examining

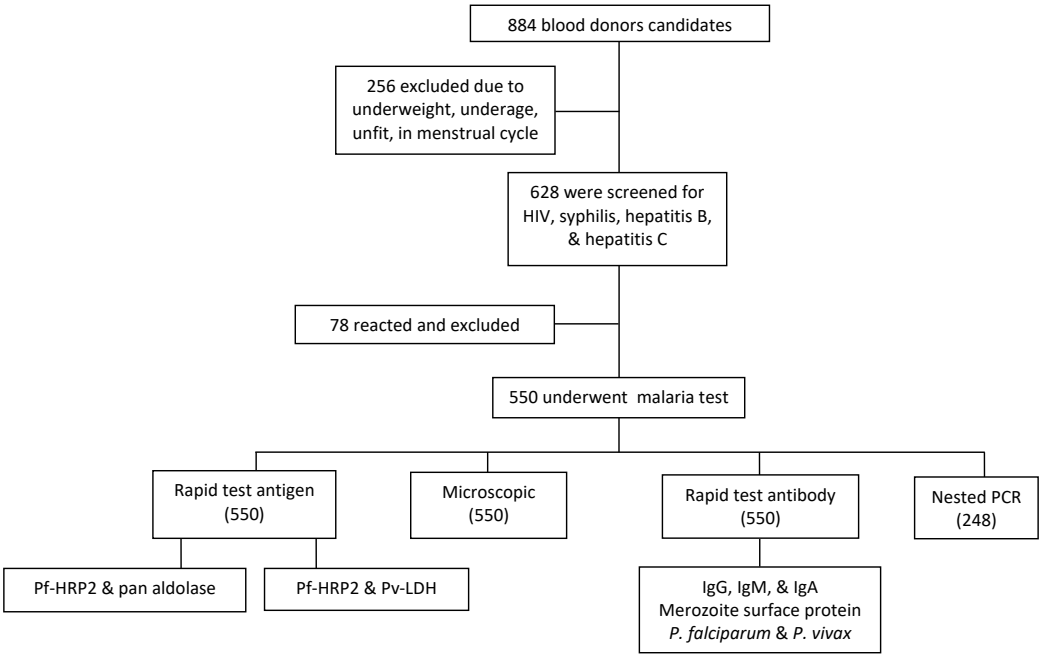

Figure 1. Trial profile. PCR=polymerase chain reaction; Pf-HRP2=*Plasmodium falciparum* histidine-rich protein-2; Pv-LDH= *Plasmodium vivax* lactate dehydrogenase

100 microscopic fields. All slides were read twice, independently in Ambon and Jakarta. A World Health Organization (WHO) certified malaria microscopist in Jakarta determined the results.

Rapid diagnostic antigens tests

Two commercial rapid antigen test kits were used, including Oncoprobe Rapid Test® (Oncoprobe, Taiwan, lot no 1303012, expired 2015) consisted of *P. falciparum* histidine-rich protein-2 (Pf-HRP2) & pan aldolase, and CTK Biotech Rapid test® (CTK Biotech Inc., USA, lot no F112311, expired 2014) consists of Pf-HRP2 & *P. vivax* lactate dehydrogenase (Pv-LDH). The procedure followed the instruction manual, in which a total of 5 µl plasma were mixed with reagents, and the results were read after 15 min (CTK Biotech) or 30 min (Oncoprobe). Positive results were considered when two or three lines appeared on the test kits.[16,17]

Rapid diagnostic antibody test

In addition, similar procedures were performed for antibody detection to identify IgG, IgM, and IgA against merozoite surface protein (MSP) of *P. falciparum* and *P. vivax*.[18] The procedures were performed according to the instruction manual of the commercial rapid test kit (Standard Diagnostic Inc., Republic of Korea, lot no 090212, expired 2014). Twenty microliters of plasma were used for the test and mixed with kit reagents for 5 to 20 min. Positive *P. falciparum* or *P. vivax* was suggested when there were two or three lines, respectively.

Polymerase chain reaction (PCR) assay

DNA was isolated from whole blood samples of each subject using conventional salting-out method.[19] PCR was carried out on the Veriti 96 well (Applied Biosystem, USA) targeting the small subunit ribosomal RNA gene (18S rRNA). The forward primer was 5-TAACGAACGAGATCTTAA-3, and the reverse primer was 5-GTTCCTCTAAGAAGCTTT-3[20] (1st BASE laboratory, Singapore). The cycle condition was 3 min at 95°C, followed by 40 cycles of 15 sec at 95°C, 15 sec at 46°C, and 15 sec at 72°C, then 1 min final extension at 72°C. Positive controls were from infected subjects (*P. falciparum*, *P. vivax*, and *P. malariae*) and buffer as the negative control. PCR products were visualized using agarose gel electrophoresis and analyzed on a UV gel doc system (Infinity, Vilber Lourmat, France). Positive PCR products were sequenced, and the basic local alignment search tool was used for species identification (https://blast.ncbi.nlm.nih.gov/Blast.cgi). PCR assay was conducted independently as the technician was blinded to the previous microscopic and rapid diagnostic tests (RDT) results.

Statistical analysis

The sample size was calculated according to the formula of single proportion for antibody detection with p = 0.3, q = 0.7, type 1 error (α) 5% (Z_α = 1.96), and d = 0.04. The minimum sample size for antibody detection was 505 specimens. However, the number of required samples for PCR assay and microscopic examination or rapid antigen test were 1,118 and 1,912, respectively. Due to financial and time constraints, we adopted sample size for antibody detection. Interim analysis was conducted in the middle of June 2013 when 302 blood samples were collected and tested. Due to negative results of any active infection for those 302 samples, the study was continued by introducing a more sensitive diagnostic test (PCR) to increase the probability of obtaining malaria evidence in the study subjects as well as at the study area. This amendment was approved by the Health Research Ethics Committee of the Faculty of Medicine Universitas Indonesia Cipto Mangunkusumo Hospital (No: 359/H2.F1/ETIK/IV/2013). Data of this study were analyzed descriptively using SPSS software version 15 (SPSS Inc., USA).

RESULTS

Of 884 candidates screened for blood donation, 256 were excluded on clinical grounds (11 had a history of clinical malaria, 29 were unfit, 77 had hyper- or hypotension, 55 had abnormal Hb levels, 22 were underweight, 18 were overweight, 26 were aged <17 years, and 18 were aged >60 years); 78 were ineligible routine laboratory screening findings (4 with HIV, 28 with hepatitis B, 25 with hepatitis C, and 21 with syphilis) (Figure 1). Thus, a total of 550 (62.2%) qualified healthy blood donors participated in this study.

Characteristics of eligible blood donors

Most blood donors were male (97.6%) and young adults (25–44 years old) (66.2%) with Hb level of >15–17 gr/dl (61.6%) and body weight of >50–70 kg (66.0%). Type O blood was the most prevalent type (56.4%), and most subjects (75%) were regular blood donors.

Laboratory examinations

The first stage of laboratory investigation was conducted on 302 blood samples, and 6.3% (19) were antibody reactive; however, none of the 302 samples were positive by microscopy or RDT antigen (Table 1). During the second stage, PCR screening was performed on 248 blood samples, which also showed negative results on microscopy and RDT antigens. However, 1.2% (3 samples: no. 358, 461, 468) of 248 samples were antibody reactive to MSP *P. falciparum* but did not show positive PCR assay. In addition, the other three samples (no. 320, 327, 362) presented bands (plasmodial DNA) on agarose gel with 200 base pairs but were not reactive with the antibody test (Figure 2 and Table 1). DNA sequencing of those amplicons was confirmed positive for *P. falciparum* in two samples: no. 320 & 327 and *P. malariae* in one sample: no. 362 (Table 2).

Thus, total seropositivity for malaria occurred among 22 of 550 (4.0%) donors examined, and positivity for plasmodial nucleic acids occurred in 3 of 248 (1.2%) donors examined. Microscopy or RDT antigen diagnostics failed to detect any sign of malaria.

DISCUSSION

A serious threat of TTM to blood product transfusion recipients indeed occurs at the transfusion service in the endemic area of eastern Indonesia that we had assessed. Serological positivity results found in the study were consistent with a recent finding of (<3 years) malaria infection incidence, i.e., it occurred in 4% of donors, while the plasmodial nucleic acid positivity occurred in 1% of donors. Traditional microscopic examination or RDT diagnostics failed to detect a single positive donor. It is likely to occur since both tests have

Table 1. Malaria positive rate based on various laboratory tests

Time period	N	Microscopic (Giemsa staining)	Rapid antigen (Pf-HRP2 and Pv-LDH)	Rapid antigen (Pf-HRP2-pan aldolase)	Rapid antibody (α-MSP-Pf Pv)	PCR 18S rRNA
First term	302	0	0	0	6.3% Pf (19)	Not done
Second term	248	0	0	0	1.2% Pf (3)*	1.2% (3)†
Total	550	0	0	0	4% Pf (22)	0.5% (3)

MSP=merozoite surface protein; PCR=polymerase chain reaction; Pf-HRP2=*Plasmodium falciparum* histidine-rich protein-2; Pv-LDH=*Plasmodium vivax* lactate dehydrogenase. *Subject no. 358, 461, and 468; †subject no. 320, 327, and 362

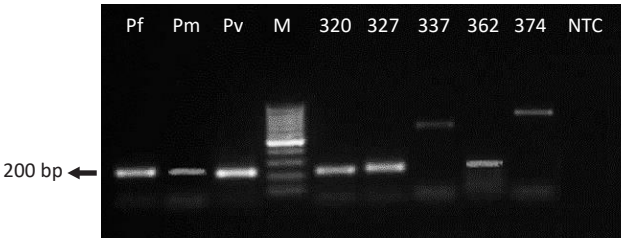

Figure 2. PCR results using allele 200 bp as targets. Lane 1: positive control *Plasmodium falciparum* 200 bp; lane 2: positive control *P. malariae*; lane 3: positive control *P. vivax*; lane 4: marker ladder DNA 100 bp PCR product; lane 5: sample no. 320 positive on 200 bp; lane 6: sample no. 327 positive on 200 bp; lane 7: sample no. 337 negative; lane 8: sample no. 362 positive on 200 bp; lane 9: sample no. 374 negative; lane 10: NTC. bp=base pair; NTC=no template control; PCR=polymerase chain reaction

lower sensitivity than PCR (microscopic and RDT=50–100 parasites/µl *versus* PCR=1–10 parasites/µl). Blood infected by plasmodial parasites and capable of causing TTM may be routinely entering the blood supplies of eastern Indonesia.

The serological test simply indicates exposure to an infection that may or may not still be active. The nucleic acid test (NAT) finding, at least theoretically, point to the presence of viable parasites and real potential for TTM. In this study, three donors (no. 320, 327, 362) were found to be positive by NAT/PCR assay, but each of them was also found negative by serological testing. Likewise, the three other donors (no. 358, 461, 468) were found to be seropositive, but none of them were proven to be NAT positive. We cannot explain why these issues may occur, but it seems that a low-grade asymptomatic parasitemia of presumed long duration might be provoking antibodies to it. The trivial explanation for it would be – the infection might have been present, but then it most likely diminished by any means. Moreover, false-negative results of PCR assay can be found in cases with low parasitemia infection and when DNA degradation occurs, i.e., repeated freeze-thaw samples.

The findings of this study still cannot be generalized as further data verification is highly required. The data should be obtained from other Blood Transfusion Units in other endemic areas with similar malaria status to Ambon Island (API<5‰). Although it is practical and inexpensive, the immunochromatographic antibody detection test needs to be further tested against different antibody test platforms, and it should be evaluated at least for sensitivity and specificity. Ideally, the reference test should be universal, and if possible, a gold standard. The WHO recommends the utilization of ELISA to screen blood donors in non-endemic countries.[21] Various productions of ELISA-based test kits have been commercially available such as DiaPro Malaria Ab®, Euroimmun anti-plasmodium ELISA®, BioRad Malaria EIA® kit, etc.[22–24] A study has compared some ELISA kits with the indirect fluorescence antibody tests (the gold standard). The study showed heterogenous sensitivity with values ranging from 53% to 64%.[22] It means that the type of test kit used will determine whether a potential donor can be

Table 2. BLAST results for species identification

No	Sample	Length/bp	Expect	Identities	DNA base sequence	BLAST hit	Access date
1.	320	180	3e-87	100%	GTG AAA GTA TATATA TAT TTT TTT ATA TGT AGA AAC TGC GAA CGG CTC ATT AAA ACA GTT ATA GTC TAC TTG ACA TTT TTA TTA TAA GGA TAA CTA CGG AAA AGC TGT AGC TAA TAC TTG CTT TAT TAT CCT GAT TTT TAT CTT TGG ATA AGT ATT TGT TAG	*Plasmodium falciparum* isolate SF1, 18S ribosomal RNA gene, partial sequence	August 1, 2021
2.	327	172	3e-75	98%	TAT ATT CTT ATT TGA ATT GAA CAT AGG TAA CTA TAC ATT TAT TCT CTA ATC AAA TTA TGA TAT TTT TAT TAA AAT ATC CTT TTC CCT GTT CTA CTA ATA ATT TGT TTT TTA CTC TAT TTC TCT TT CTT TTA AGA ATG TAC TTG CTT GAT TGA AAA GCT TCT TAG AGG	*Plasmodium falciparum* genome assembly, chromosome: 7	August 1, 2021
3.	362	210	7e-104	100%	TAG TCA TAT GCT TGT CTC AAA GAT TAA GCC ATG CAA GTG AAA GTA TAT GCA TAT TTT ATA TGT AGA AAC TGC GAA CGG CTC ATT AAA ACA GTT ATA GTC TAC TTG ACA TTT TTT TTA TAA GGA TAA CTA CGG AAA AGCTGT AGC TAA TAC TTG CTT TAA TAC TCT TAA TTC TTT ATG TTT TTT GAG TAT GTA TTT GT	*Plasmodium malariae* rRNA (PmUG01_03031900)	August 1, 2021

BLAST=basic local alignment search tool; bp=base pair

included or excluded. Although the assurance of blood transfusion recipient safety with respect to TTM may be impractically difficult, the exclusion of seropositive donors may be the most pragmatic approach. On the other hand, using PCR assay as a routine screening is not feasible due to the high operational cost. Fever monitoring in individuals receiving blood transfusion should be carried out during and after a transfusion session. In addition, malaria microscopic or rapid diagnostic antigen testing should be performed whenever fever occurs.

There are two other studies related to the diagnosis of malaria among blood donors in Indonesia. The first study was conducted at the Blood Transfusion Unit of Padang city, West Sumatra involving 97 subjects, and the diagnostic test was performed with rapid mono antigen tests.[25] Similar to our findings, the study reported that none of the subjects had a positive result. Meanwhile, the second study was conducted at the Blood Transfusion Unit in Sikka, Flores.[26] The study used RDT antigen and microscopic examinations. However, there was no data regarding the positivity rate of the candidate blood donors. Although this study is not the first laboratory study conducted on blood donors living in malaria-endemic areas of Indonesia, the strength of this study is the most comprehensive laboratory study of malaria in Indonesian blood donors. It also demonstrated the risk of TTM occurrence in a blood transfusion unit setting.

The limitation of this study is the unmet sample size to assess the sensitivity of microscopic Giemsa examination and rapid antigen test in blood donor candidates. Both diagnostic tests are usually performed routinely to confirm malaria diagnosis in symptomatic individuals. There were 11 excluded candidates by interviews on the history of fever, which seems that a routine clinical judgment, and suspected history of clinical malaria are good enough to eliminate acute malaria-infected individuals. Conceivably, residents in the study area have not yet developed their clinical immunity to the infections. Therefore, further studies using a larger sample size might eventually provide similar results of no malaria positive detected on using microscopic and RDT antigen tests. The results of the study may have an impact on safe blood transfusion; therefore, further development on the effective malaria screening strategies are needed to reduce the risk of TTM, which provides better surveillance on blood donor diseases and ensures a safe blood transfusion therapy. In conclusion, around 4.5% (25/550) of blood donor candidates were reactive to the laboratory tests; therefore, performing malaria tests is essential and necessary to prevent TTM in an endemic area of Indonesia.

Conflict of Interest

Inge Sutanto is the editorial board member but was not involved in the review or decision process of the article.

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