Original Article

Seroprevalence of malaria in blood donors and multi-transfused patients in Northern India: Relevance to prevention of transfusion-transmissible malaria

Anju Dubey, Priti Elhence, Ujjala Ghoshal[1], Anupam Verma

Departments of Transfusion Medicine, and [1]Microbiology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India

Abstract:

Background: Transfusion-transmissible malaria (TTM) is a major concern in malaria endemic countries. A study was therefore conducted to know sero-prevalence of malaria in blood donors and the risk of TTM to multi-transfused patients at our hospital. **Materials and Methods:** Study subjects were: eligible blood donors (n = 1000), donors deferred due to history of fever in the last 3 months (n = 100), and multi-transfused patients (n = 200). Screening for malaria was done by slide microscopy, immunochromatographic rapid diagnostic test (RDT) for malaria antigen, and anti-malaria antibody by enzyme linked immunosorbent assay. **Results:** Malaria antibody prevalence in eligible donors and donors with history of fever, thalassemia patients, and in other multi-transfused patients was 16.9%, 22%, 6%, and 15%, respectively. None of the donors were positive for malaria on microscopic examination. None of the blood donors except one donor with history of fever, tested positive with RDT. **Conclusion:** Malaria antibody prevalence in blood donors at our center is high. As blood units donated by such donors have high-risk potential, special processing may be undertaken to reduce the risk of TTM.

Key words:

Anti-malaria antibody, blood donor, multi-transfused patients, serology, transfusion-transmitted malaria

Access this article online

Website: www.ajts.org

DOI: 10.4103/0973-6247.98937

Quick Response Code:

Correspondence to:
Dr. Priti Elhence,
Department of Transfusion Medicine, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow – 226 014, Uttar Pradesh, India.
E-mail: pelhence@sgpgi.ac.in

Introduction

Vector-borne malaria is a major public health problem in India; however, the malaria endemicity is quite variable across the country.[1] The annual parasite incidence (API) from our region is reported to be <2 per 1000 population whereas regions with API >5 per 1000 are scattered in the states of Rajasthan, Gujarat, Karnataka, Goa, Southern Madhya Pradesh, Chhattisgarh, Jharkhand, Orissa, and North Eastern States.[1]

In malaria endemic countries, transfusion transmitted malaria (TTM) can be a significant problem because of certain characteristics of malaria infection, i.e.,: (a) Semi-immune individuals with low level of parasitemia remain asymptomatic and can qualify as blood donors, (b) *Plasmodia*, the malarial parasite, is able to survive in blood stored at 4°C, and (c) The sensitivity of currently used methods for malaria screening (Microscopic examination: ~ 50 parasites/μL; rapid diagnostic device (RDT): ~ 100 parasites/μL) is much lower than that required to detect level of parasitemia capable of causing TTM (~ 0.00004 parasites/uL or 1-10 parasites/unit of blood).[2]

In India, strategies adopted to prevent occurrence of TTM are: a) mandatory deferral of donors with history of fever (presumably malaria) in the last 3 months and b) to test donated blood for presence of malaria infection.[3] However, prevalence of markers for malaria in blood donors and incidence of TTM in patients is scantily reported, though significant risk is highlighted by frequent case reports.[4-7] Therefore, it is prudent to know the prevalence of malaria in local donor population and usefulness of currently adopted prevention strategies.

Antibodies to all four *Plasmodium species* are produced 1 to 14 days after initial infection.[3] Semi-immune malaria high-risk donors can be identified by malaria antibody screening by enzyme immunoassays (EIA), which are now available commercially. These assays provide a more sensitive and practical alternative to identify malaria high-risk donors.

A pilot study was therefore undertaken at our center to study prevalence of malaria antigen and antibody in eligible blood donors, in donors excluded on the basis of history of fever in last 3 months and in multi-transfused patients to assess the risk of TTM and usefulness of currently adopted preventive strategies.

Materials and Methods

This retrospective, cross-sectional study was conducted at the transfusion service of a tertiary care teaching hospital in the state of Uttar Pradesh in Northern India, from October 2006 to August 2008. It was approved by our Institute's research and ethics committee. Informed consent was taken from all subjects included in the study.

Subjects and Samples

Study population consisted of 1000 randomly selected eligible blood donors with no history of fever in the past 3 months; 100 deferred donors due to history of suspected malaria in the past 3 months, and 200 multi-transfused patients (thalassemia patients n = 100, others n = 100) who had been transfused >10 units of packed red blood cells (PRBC) in the past 1 year. The demographic, transfusion, and other clinical details of donors and patients were recorded from blood donor cards, case files, and computer-based hospital information system.

At the time of inclusion in the study, 2 mL of blood sample in Ethylenediaminetetra-acetic acid (EDTA) vial and 5 mL of plain blood sample were collected from the subjects. EDTA sample was used for microscopic slide study and malaria antigen testing by RDT. Serum was separated from plain sample and preserved at -20°C for malaria antibody testing by enzyme linked immunosorbent assay (ELISA).

Malaria testing

Microscopic examination for malaria parasite was done by thick and thin smear examination using standard methods.[8] A thick smear was drawn, stained with Giemsa stain, and observed under microscope in low power, high power, and then using oil immersion lens. If positive, a thin smear was made for species identification. In addition, all samples were also tested for malaria antigen and anti-malaria antibodies. Malaria antigen testing was done on EDTA blood samples by RDT device, which is a pan malaria test based on detection of malaria parasite-specific lactate dehydrogenase (pLDH) (PARABANK, Zephyr Biomedicals, Goa, India) as per the manufacturer's instructions. Results were indicated by the presence or absence of a band in the test region. Malaria antibody testing was done by commercially available malaria antibody ELISA (Pan Malaria Antibody CELISA, Cellabs Pty Ltd. Brook vale, Australia), which detects specific IgG antibody against *P. falciparum, P. vivax, P. malariae,* and *P. ovale*. Tests were done as per the manufacturer's instructions. Samples with optical density above the cut-off value were labeled as positive.

Malaria antibody prevalence was compared among study subjects. Metselaar and van Theil criteria were used to categorize the study population on the basis of anti-malaria antibody prevalence as hypo-endemic (<10%), meso-endemic (11-50%), hyper-endemic (51-75%), and holo-endemic (>75%).[9] Correlation of antibody prevalence in blood donors in relation to gender, type of donor, frequency of donation, zone, and area of residence was also done. Malaria antibody positive and negative patients were compared with respect to age; number of PRBC transfusions received in the defined period, effect of splenectomy, or occurrence of splenomegaly to study any correlation.

Data was maintained on SPSS version 13 and Chi Square tests were applied to explore differences in antibody prevalence on the basis on donor characteristics. Student t test was used to compare the means of two variables for a single group. A *P* value of less than 0.05 was considered significant.

Results

Majority of eligible blood donors were males (93.2%), replacement donors (95.9%), urban (90.6%), residents of non-endemic zones (96.5%), and donating blood for the first time (72.5%). There were no demographic differences between the eligible and deferred blood donors. None of the eligible (n = 1000) or deferred (n = 100) blood donors were positive for malaria by slide microscopy. None of the selected donors were positive for malaria antigen by RDT; however, one of the deferred donors with recent history of fever (1%) was positive for malaria antigen by RDT. Thus, overall malaria antigen prevalence in blood donors was 0.09%. This donor was also positive for anti-malaria antibody by ELISA.

Malaria antibody prevalence in blood donors

One hundred and sixty-nine (16.9%) of the eligible donors were reactive for anti-malaria antibody as compared to 22 (22%) of deferred donors with history of fever, though this difference was not statistically significant. The overall malaria antibody prevalence was 17.4%, and thus, donor population in our region was found to be meso-endemic for malaria. The demographic characteristics of blood donors and prevalence of anti-malaria antibodies are summarized in Table 1. No statistically significant difference in seropositivity was evident between replacement and voluntary donors, first time and repeat donors, donors residing in non-endemic zones and those residing in endemic zones (P >0.05). However, there was significantly high prevalence of anti malaria antibody in rural donors as compared to urban donors (P = 0.001).

Malaria antibody prevalence in multi-transfused patients

Malaria antibody prevalence in thalassemia patients (6%) was much lower than in other multi-transfused patients (15%); however, the difference was not statistically significant (P >0.05). As shown in Table 2 there was no significant difference between mean age and number of PRBC units transfused in the last

Table 1: Malaria antibody prevalence in relation to donor demographics in selected donors (*n* = 1000)

Donor parameter	No. of donors Total (N)	Malaria antibody prevalence n (%)	*P*
Gender			
Males	932	158 (17)	0.514
Females	68	11 (16.2)	
Type of donor			
Replacement	959	162 (16.9)	0.555
Voluntary	41	07 (17.1)	
Frequency of donation			
First time	725	125 (17.3)	0.358
Repeat	275	44 (16)	
Zone of residence			
Non-endemic	965	164 (17)	0.443
Endemic	35	05 (14.3)	
Area of residence			
Urban	906	138 (15.2)	0.001*
Rural	94	31 (33)	

**P* value significant

5 years among anti-malaria antibody reactive and non-reactive thalassemia patients. A higher percentage of patients (33.3%) had been splenectomized among those reactive for anti-malaria antibodies as compared with non-reactive group (12.8%); however, the difference was not significant statistically. Among the other multi-transfused patients group also, there was no difference between the mean age, mean PRBC transfusion in the last 1 year, and presence of splenomegaly between malaria antibody non-reactive and reactive patients.

Table 2: Comparison between anti-malaria antibody reactive and non-reactive multi-transfused patients (n = 200)

Patient category	Parameter	Malaria antibody reactive	Malaria antibody nonreactive	*P* value
1. Thalassemia patients (*n* = 100)	*n*	06	94	
	Age (years) Mean ± SD	11.6 ± 5.47	13.3 ± 6.3	0.459
	No. of PRBC transfusion in last 5 years (Mean ± SD)	96.3 ± 38.4	100.3 ± 46.5	0.835
	Splenectomized *n* (%)	2 (33.3)	12 (12.8)	0.197
2. Other multi-transfused patients (*n* = 100)	*n*	15	85	
	Age (years) Mean ± SD	41.3 ± 17.7	47.7 ± 22.9	0.221
	No. of PRBC transfusion in last 1 year (Mean ± SD)	14.7 ± 7.6	15.7 ± 11.1	0.687
	Splenomegaly (%)	2 (13.3)	12 (14.1)	0.936

During the study period, two thalassemia patients developed malaria: one was caused by *P. falciparum* and other by *P. vivax*. Both of these patients had received PRBC transfusion two weeks prior to the malaria episode and these units were found to be malaria antibody positive on retrospective testing of donor samples. None of the other multi-transfused patients were positive for malaria by either slide microscopy or RDT.

Discussion

On the basis of overall malaria antibody prevalence (17.4%) in blood donors, our region can be categorized as meso-endemic for malaria. In a study done by Choudhry *et al*. in North Indian blood donors more than a decade ago, malaria antibody was detected in 12.39% and 19.37% of subjects by Indirect Fluorescence Antibody test (IFAT) and in-house ELISA, respectively.[10] Our results compare well with their study, as at that time the history-based

Table 3: Malaria antibody and antigen prevalence in blood donors from India and other countries

	Author	Country	Study group (n)	Malaria antibody prevalence %	Method	Malaria antigen prevalence %
Endemic areas	Choudhury N *et al*.[10]	India	Voluntary blood donors	12.39	Indirect fluorescent antibody test	0.35
				19.37	ELISA	(Monoclonal antibody technique)
	Achidi *et al*.[11]	Nigeria	Blood donors (416)	100	Indirect fluorescent antibody test	ND*
	Lim CS *et al*.[12]	Korea	Blood donors	6.7	Indirect Fluorescence Antibody test	1.7 (PCR)
	Sáez-Alquézar *et al*.[13]	Brazil	Blood donors	80.6	Indirect Immunofluorescence test	None (Immunofluorescence test)
	Contreras *et al*.[14]	Venezuela (Boliver)	Blood donors (1000)	3.8	Indirect immunofluorescent antibody	ND*
				2	ELISA	
	Saeed *et al*.[15]	Saudi Arabia	Voluntary blood donors (1756)	7.6	ELISA	0.17 (ELISA)
	Amer *et al*.[16]	Qatar	Voluntary blood donors (5845)	4.3	Indirect Immunofluorescence test	ND*
	Diop *et al*.[17]	Senegal	Blood donors (3001)	65.3	ELISA	0.53 (ELISA)
	Present study	India	Blood donors (1100)	17.4	ELISA	0.09 (immunochromatographic test)
Non endemic areas	Chiodini *et al*.[8]	U.K	Blood donors (5311)	0.45	ELISA	ND*
	Contreras *et al*.[14]	Venezuela (Caracas)	Blood donors (1000)	0.8	Indirect immunofluorescent antibody test	ND*
				0.8	ELISA	
	Soler *et al*.[18]	France	Blood donors (276)	1.62	Indirect Immunofluorescence test	ND*
	Seed *et al*.[19]	Australia	Malaria exposed blood donors (751)	1.33	Enzyme immunoassay	ND*

*Not done

donor deferral for malaria was not followed. Table 3 summarizes the malaria antibody prevalence in blood donors reported from various endemic and non-endemic countries and strategies adopted to prevent TTM, for comparison. As seen in Table 3, malaria antibody screening of blood donors is a routine method to prevent TTM in non-endemic countries. However, since malaria antibody prevalence in our donor population is high, discarding of blood on the basis of malaria antibody positive result is not a feasible option. In a study done by Oh *et al.* malaria antibody ELISA was found to have a clinical specificity of 94.0% for *P. vivax* with polymerase chain reaction (PCR) as reference method.[20] Thus, it would be prudent to evaluate and adopt additional strategies to make these units non infectious.

The statistically insignificant higher seroprevalence of malaria antibody in donors having history of fever within the last 3 months (22%) as compared with that in normal donors (16.9%) does not provide enough evidence at this stage to prove or disprove usefulness of such criteria, and results need to be confirmed on a larger sample size study to prevent unnecessary donor deferrals. Except for rural residence (33% vs. 15.2% in urban) no other donor characteristics studied, i.e., age, gender, or type of donor, had any bearing on malaria antibody prevalence. This is in concurrence with reported findings and is closely related to the agricultural practices and habits such as sleeping out of the doors and not using measures of personal protection.[21]

None of the donors was found to be positive for malaria by microscopy or RDT expect one deferred donor (0.09%) who tested positive with RDT, while in a study done by Bahadur *et al.* recently, 0.03% out of 11,736 units of donated blood were positive for malaria by RDT.[22] Therefore, blood donor screening for malaria by microscopy may not be an acceptable method as more sensitive malaria screening methods like RDT and malaria antigen testing by ELISA are now available.

Malaria antibody prevalence in multi-transfused patients was not greater than in blood donors. Therefore, no conclusion can be made as to whether malaria exposure through transfusion is a significant risk factor. Rather, the prevalence of malaria antibody in thalassemia patients (6%) was considerably lower as compared with that in donor population (17.4%), though not statistically significant. The difference could be because of lesser duration of exposure to community-acquired vector borne malaria, as majority of the thalassemia patients (90%) were less than 18 years of age whereas all the donors were above 18 years. Other studies have reported malaria incidence of 6.4%[6] and 6.9%[23] in thalassemia patients. In contrast, patients with Hb E- β Thalassemia disease at the National Thalassemia Center in Kurunegala, Sri Lanka, a region of low malarial transmission, have been found to have high frequencies of antibodies to *P. vivax* (>60%) and to a lesser degree to *P. falciparum* (>30%) from the early years of life, and the levels are significantly higher than those of age-matched controls from the same region, suggesting increased susceptibility.[24] The same study also reported significantly higher malaria antibody prevalence in thalassemics with splenomegaly or those who have undergone splenectomy. This finding was also not confirmed in our study, and the issue needs further investigation by comparing antibody prevalence in healthy non-transfused and transfused age-matched controls.

Malaria antibody prevalence in other multi-transfused group of patients in our study was 15%, which was not significantly different from the normal healthy donors acquiring malaria by vector. In comparison, in a study done by Ali *et al.* in 2004, post transfusion malaria incidence of 4.9% has been reported for multi-transfused patients.[25]

As with other transfusion-transmitted infections, suspected TTM was difficult to prove to be transmitted by transfusion, as implicated donors did not report for follow-up despite repeated requests.

In conclusion, the existing strategy of donor deferral for fever in preceding 3 months can be combined with anti-malaria antibody screening by commercially available ELISA. Anti-malaria antibody positive units may then undergo pathogen inactivation to render them non-infectious before transfusion or anti-malaria chemoprophylaxis can be given to recipients of anti-malaria antibody reactive units as targeted intervention. The ideal approach, however, would be to screen all donations for malaria by PCR which is currently the most sensitive technique (~ 5 parasites/ uL).[26] A recently available technique based on detection of hemozoin pigment in the neutrophils and monocytes by automated hematology cell counters should also be evaluated as it is a convenient, less costly, and objective method.[27] The usefulness of each, however, has to be evaluated in terms of TTM cases prevented and the additional costs incurred.

References

1. Kumar A, Valecha N, Jain T, Dash AP. Burden of malaria in India. Retrospective and prospective view. Am J Trop Med Hyg 2007;77:69-78.
2. Malik V. Drug & Cosmetics Act of India 1940. 13th ed. Lucknow (India): Eastern Book Company; 2001.
3. Seed C, Kitchen A, Davis TM. The current status and potential role of laboratory testing to prevent Transfusion Transmitted Malaria. Transfus Med Rev 2005;19:229-40.
4. Mahapatra AK, Banerji AK. Transfusion malaria amongst operated neurosurgical patients-a prospective study of 22 cases. Indian J Malariol 1989;26:91-4.
5. Mahapatra AK, Rao DN. Transfusion malaria in operated neurosurgical cases: A report of two cases. Indian J Malariol 1990;27:243-5.
6. Choudhury NJ, Dubey ML, Jolly JG, Kalra A, Mahajan RC, Ganguly NK. Post-transfusion malaria in thalassaemia patients. Blut 1990;61:314-6.
7. Agarwal S, Maini S, Kelkar AA, Tijare JR, Shrikhande AV. Probable transfusion transmitted malaria in child of sickle cell disease (HbSS). Indian J Pediatr 2008;75:646.
8. Chatterjee KD. Malarial parasites of man, In: Chatterjee KD, editors. Parasitology in relation to Clinical Medicine. 12th ed. Calcutta: Chatterjee Medical Publishers; 1995. p. 215-6.
9. Metselaar D, van Theil PM. Classification of malaria. Trop Geogr Med 1959;11:157-61.
10. Choudhury NJ, Jolly JG, Mahajan RC, Dubey ML, Ganguly NK, Agnihotri SK. Donor Screening for malaria by antibody and antigen detection in endemic area. Indian J Malariol 1991;28:179-82.
11. Achidi EA, Perlmann H, Berzins K. Asymptomatic malaria parasitaemia and seroreactivities to *Plasmodium falciparum* antigens in blood donors from Ibadan, South-western Nigeria. Ann Trop Med Parasitol 1995;89:601-10.
12. Lim CS, Kim YK, Lee KN, Lee HW, Lee WJ, Lee JS, *et al*. Malaria detection rate of donated blood and blood sample in risky area. Korean J Blood Transfus 1997;8:103-11.

13. Sáez-Alquézar A, Ramos AM, Di Santi SM, Branquinho MS, Kirchgatter K, Cordeiro IA, *et al*. Control of blood transfusion malaria in an endemic and in a non-endemic region in Brazil. Rev Soc Bras Med Trop 1998;31:27-34.
14. Contreras CE, Pance A, Marcano N, González N, Bianco N. Detection of specific antibodies to *Plasmodium falciparum* in blood bank donors from malaria-endemic and non- endemic areas of Venezuela. Am J Trop Med Hyg 1999;60:948-53.
15. Saeed AA, Rasheed AM, Naseer IA, Onaizi MA, Kahtani SA, Dubois L. Malaria screening of blood donors in Saudi Arabia. Ann Saudi Med 2002;22:329-32.
16. Amer A, Al Malki A, Yousef B, Al Mutawa H. Improved screening of donors blood for malaria: A Qatar experience. Qatar Medical J 2005;14:23-5.
17. Diop S, Ndiaye M, Seck M, Chevalier B, Jambou R, Sarr A, *et al*. Prevention of transfusion transmitted malaria in endemic area. Transfus Clin Biol 2009;16:454-9.
18. Soler CP, Gerome P, Soullie B, Tissedre F, Bejan H, Yvetot J, *et al*. Comparison of techniques used to screen blood donations for malaria antibodies. Med Trop (Mars) 2003;63:587-9.
19. Seed C, Cheng A, Davis TM, Bolton WV, Keller AJ, Kitchen A, *et al*. The efficacy of a malarial antibody enzyme immunoassay for establishing the reinstatement status of blood donors potentially exposed to malaria. Vox Sang 2005;88:98-106.
20. Oh JS, Kim JS, Lee CH, Nam DH, Kim SH, Park DW, *et al*. Evaluation of a malaria antibody enzyme immunoassay for use in blood screening. Mem Inst Oswaldo Cruz 2008;103:75-8.
21. Martens P, Hall L. Malaria on the move. Human population movement and malaria transmission. Emerg Infect Dis 2000;6:103-9.
22. Bahadur S, Pujani M, Jain M. Use of rapid detection test to prevent transfusion transmitted malaria. Asian J Transfus Sci 2010;4:140-1.
23. Harano K, Than AM, Harano T, Win T. Malaria parasites detected in Myanmar thalassemia patients. Kawasaki Med J 2002;28:17-20.
24. O'Donnella A, Premawardhena A, Arambepolad M, Samaranayaked R, Allenb SJ, Petob TE, *et al*. Interaction of malaria with a common form of severe thalassemia in an Asian population. Proc Natl Acad Sci U S A 2009;106:18716-21.
25. Ali MS, Kadaru AA, Mustafa MS. Screening blood donors for malaria parasite in Sudan. Ethiop J Health Dev 2004;18:70-4.
26. Benito A, Rubio JM. Usefulness of seminested polymerase chain reaction for screening blood donors at risk for malaria in Spain. Emerg Infect Dis 2001;7:1068.
27. Scott CS, Zyl DV, Ho E, Meyersfeld D, Ruivo L, Mendelow BV, *et al*. Automated detection of WBC intracellular malaria – associated pigment (Hemozoin) with Abbott Cell DYN 3200 and Cell DYN 3700 Analysers: Overview and results from South African institute for medical research (SAIMR) II evaluation. Lab Hematol 2002;8:91-101.

Cite this article as: Dubey A, Elhence P, Ghoshal U, Verma A. Seroprevalence of malaria in blood donors and multi-transfused patients in Northern India: Relevance to prevention of transfusion-transmissible malaria. Asian J Transfus Sci 2012;6:174-8.

Source of Support: Nil, **Conflict of Interest:** None declared.