STUDIES OF A MALARIA ANTIGEN ELISA FOR SCREENING OF BLOOD DONORS: ANTIGEN CARRIAGE AND RISK OF TRANSFUSION MALARIA

M. de S. WIJESUNDERA, R.L.A.R. RANAWEERA, N. EKANAYAKE* AND C. JAYASINGHE

Department of Parasitology, Faculty of Medicine, University of Peradeniya and **Haematology Unit, General Hospital, Kandy.*

ABSTRACT

The persistence of post-treatment antigenaemia in falciparum malaria and the risk of transfusion malaria with *Plasmodium falciparum* were evaluated using an ELISA, based on *Pf* HRP2 antigen capture assay. In 33 falciparum patients treated with chloroquine and followed up, 27(81%) were positive for antigenaemia on day 3, and 12 (38%) remained positive on day 14 suggesting the extent of drug resistant falciparum prevalence in this community.

The study did not show a risk of falciparum-transfusion malaria in multiple transfusion recipients. in the General Hospital, Kandy.

It is concluded that thick blood film screening of donor bloods is an effective method to limit risk of transfusion malaria in Sri Lanka.

INTRODUCTION

Malaria can be transmitted by the transfusion of blood or products likely to contain even a small number of red cells . An inoculum containing as few as 10 parasites is sufficient to cause transfusion malaria (Bruce-Chwatt, 1972). Parasites remain viable in stored blood for at least a week but cases of *Plasmodium vivax* malaria have been reported from blood stored even up to 19 days. However, overall, the risk of transfusion malaria is highest with infected blood transfused within 5 days of storage (Mollison, Engelfriet & Contreras, 1987).

Post transfusion malaria is rare in developed countries due to stringent exclusion of blood donors on past history and serology. But in many malarious countries the extent of the risk is unknown, largely due to the lack of reporting. Until recent times transfusion malaria was not particularly a therapeutic problem as chloroquine, a cheap, safe and effective drug was widely used to treat both donor and recipient. However, with the rapid spread of multidrug resistance globally, the risk of transfusion malaria with *Plasmodium falciparum* is a serious health hazard especially so in an already ill patient requiring blood.

In Sri Lanka, Wickramasinghe (1976) reported a series of 16 cases of transfusion malaria occurring during 1960-1966 at a time when malaria was near eradication. Of the total, 14 were *P malariae* infections and 2, *P falciparum.* Since then, although there has been a resurgence of malaria in Sri Lanka with several major epidemics, transfusion malaria is not reported or investigated, especially in view of the

fact that currently the dry zone and much of the intermediate zone remain malarious. Furthermore, parasite carriage in blood donors has been documented in Sri Lanka. Nageswaran *et al,* in 1987 showed a 6.9% malarial parasite rate by microscopy in 246 donors in the blood bank in General Hospital, Jaffna. A study carried out by us in 1994-1995 in screening 1103 donor bloods from Kandy and Peradeniya blood banks, showed a 9.5% malaria antibody carriage and in the positives, a high degree (79%) *P falciparum* antigen carriage using a microplate ELISA assay (Wijesundera *et al,* 1996). This test detects a soluble parasite protein viz. histidine rich protein-2 (HRP-2) of *P falciparum* rather than the parasites themselves. One of the limitations of this assay is the persistence of antigenaemia after treatment and clearance by microscopy (Namsiripongpun *et al,* 1993).

Thus the objectives of this study were:

1. to determine post treatment antigen carriage in falciparum malaria.

2. to assess risk of transfusion malaria with *P falciparum* in multiple transfusion patients at the General Hospital, Kandy.

MATERIALS & METHODS

Persistence of post treatment antigenaemia with *Pf* HRP2

Study group

This consisted of patients admitted to General Hospitals, Kurunegala and Badulla during 1995-97 diagnosed as falciparum malaria on a thick blood film positive for asexual parasites and who were treated with a standard regimen of chloroquine.

A total of 68 patients were initially included, but of these 33 testing positive on serology and microscopy were followed up for 14 days post treatment. Blood samples were taken at day 0, before drug treatment and at day 3 and day14 post treatment. Those with mixed infections, *P vivax* infections and those having history of prior antimalarial therapy, were excluded.

Risk of post transfusion malaria in multiple transfusion patients.

Study group

This consisted of patients treated at the Haematology Unit General Hospital, Kandy receiving transfusions during October '96 to April '97. Of the 112 transfused during this period, 88 were included in the study. Blood sampling was done on day 0 (pretransfusion DT sample was used) and on day 14. All blood samples were stored at -20 ^{0}C.

Information on number of transfusions received during the last two years, details of current transfusion, malaria exposure risk, residency in malaria endemic areas and prior treatment with antimalarials were obtained from each patient using a questionnaire.

ELISA assay for *Pf* HRP2 antigen

Malaria Ag ELISA was carried out with microplates coated with IgM Mab to *Pf* HRP2 (Voller, Bidwell and Chiodini, 1994). Tests were carried out with 100µl of lysed whole blood , peroxide conjugated antihuman IgG, substrate Tetramethylbenzidine (TMB) and read at 450 nm. The cut off level was taken as the Reference Negative reading +0.05.

RESULTS

Antigen carriage in *Pf* HRP2 positive patients following standard regimen of chloroquine is shown in Table I.

Table I . Antigen carriage in *Pf* HRP2 positive patients treated with chloroquine

DAY	D0	D3 (n=32*)	D14 (n=22)
ELISA positivity	+ -	+ -	+ -
Number	33 0	27 05 (81%)	12 10 (38%)
Blood film (*Pf* asexual stages)	Positive	Negative	Negative

*1 Death

The 88 multiple transfused patients consisted of 45 males and 47 females. The ages ranged from 1-78 years with a median age of 39 years. The number of transfusions received by the recipients during the last 2 years and the blood storage time of the donor blood is shown in Tables II and III.

One recipient developed fever one week after transfusion but was negative for malaria parasites by microscopy and for *Pf* antigen carriage. Three had received prior antimalarials either as prophylaxis or for treatment. Only one patient's blood showed a positive sero-conversion but this patient's pre-transfusion blood was also positive and although she denied previous malaria attacks she was a resident of Vavuniya, a malaria endemic area.

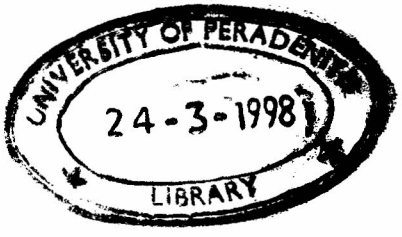

Table II. Blood transfusions received per recipient during last two years

umber of transfusions	No. recipients
< 5	53 (60%)
5-19	30
≥20	05
Total	88

Table III. Duration of donor blood storage before transfusion

Days	No. transfusions
≤ 5	11 (35%)
6-14	08
≥14	12
Total	31

DISCUSSION

Persistence of antigeneamia

In *P falciparum* assays based on HRP 2 Ag. capture, with both micro ELISA and the more recent rapid dipstick methods, a limitation in interpretation of results is the persistence of antigen as late as 7-14 days after clearance by microscopy (Namsiripongpun *et al*, 1993). Our study shows a 38% persistence at day 14 following chloroquine therapy. These results are in agreement with those of two recent studies carried out in the region.

Singhe, Valecha & Sharma (1997) carrying out a field study in central India using the ICT Malaria Pf Test™ reported a 30% persistence of antigen carriage at 7 days following treatment with sulphadoxine-pyremethamine while Kodisinghe *et al* (1997) during a study in Kurunegala-Monaragala using the *Para*Sight™-F dipstick technique, followed up 15 patients with parasitaemia of over 1500 per µl. They found persistence of antigenaemia in all the patients at day 3 and in 2 patients, persistence of antigenaemia at day 14. The latter patients were subsequently confirmed to have recrudescence of malaria. Therefore antigen positivity probably reflects the prevalence of chloroquine resistant falciparum.

Falciparum malaria risk in multiple transfusion patients

Although theoretically transfusion with asexual parasites could give rise to clinical infections soon after, invariably the low donor -parasitaemia requires multiplication over

several cycles to cause clinical disease. The incubation period of post transfusion malaria depends on the parasite inoculum as well as the species of parasites transfused. For *P falciparum* and *P vivax*, it is between 1 week to 1 month, while for *P malariae* it may be several months (Mollison, Engelfriet and Contreras, 1987)). In this study in assaying the risk of post transfusion falciparum malaria, blood was sampled fortnightly. However, this sampling might not be adequate and may miss *P falciparum* infections having longer incubation periods.

Currently at the blood bank General Hospital Kandy, all donor bloods are screened microscopically with thick films by a trained technician of the blood bank. This screening had detected two positive donors during the last two -year period but both were *P vivax* infections (Mr. M.B. Chandrasiri, personal communication).

Thus it is very likely that this routine screening procedure effectively limits the risk of transfusion malaria and further has the advantage that it covers all parasite species unlike the antigen detection tests which currently are limited to detecting *P falciparum*. It is noteworthy that Kodisinghe *et al* (1997) concluded that there was no significant difference in sensitivity or specificity between the *Para*Sight ™ -F dipstick test and thick film microscopy by trained technicians in detecting falciparum infections.

Yet another protective measure recommended to limit risk of transfusion malaria is the use of stored blood of more than 14 days. However, under certain disease conditions such as in patient care undertaken in haematology units, fresh blood needs are high as shown in this study. Therefore this protective measure may not always be applicable.

CONCLUSIONS

The persistence of antigenaemia at 14 days suggests a high degree of drug resistant falciparum malaria in these areas. The study did not show a risk of post transfusion falciparum malaria in-patients receiving multiple transfusions and it is concluded that thick blood filming of donor bloods by a trained technician is an effective strategy to limit risk of transfusion malaria in hospitals in Sri Lanka.

ACKNOWLEDGMENTS

We thank Drs. M.P. Wedisinghe and A. Jayaratne for assistance given in the Kurunegala study and Dr. J.K.K. Seneviratne for assisting us in the Badulla study. This study was funded by University of Peradeniya Research Grant RG/96/40/M.

REFERENCES

Bruce- Chwatt, L.J. (1972) Blood transfusion and tropical disease. *Tropical Diseases Bulletin*, **69**, 825-862

Kodisinghe, H.M., Perera, K.L.R.L., Premawansa, S., Naotunne,T. de S., Wickramasinghe, A.R. and Mendis, K. N. (1997) The *Para*Sight™ -F dipstick test as a routine diagnostic tool

for malaria in Sri Lanka. *Transactions of the Royal Society of Tropical Medicine & Hygiene*, **91**, 398-402

Mollison,P., Engelfriet,C.P.and Contreras,M. (1987) in Blood Transfusion in Clinical Medicine. Blackwell Scientific Publications, Oxford. pp 801-804.

Namsiripongpun,V.,Wilde,H., Pamsanduan,P. and Tiersansern,P. (1993) Field study of an antigen detection ELISA specific for *Plasmodium falciparum* malaria . *Transactions of the Royal Society of Tropical Medicine & Hygiene*, **87**, 32-35.

Nageswaran, A., Nageswaran, C., Suthaharan, K. and Phillipupillai, M.J.R. (1987) Malarial parasite rates among blood donors. *Jaffna Medical Journal*, **22**, 48-49.

Singhe,N.,Valecha, N.and Sharma,V.P.(1997) Malaria diagnosis by field workers using an immunochromatographic test. *Transactions of the Royal Society of Tropical Medicine & Hygiene*, **91**, 396-397.

Voller, A., Bidwell, D.E. and Chiodini, P.L. (1994) Evaluation of a malaria antigen ELISA. *Transactions of the Royal Society of Tropical Medicine & Hygiene*, **88**, 188.

Wickramasinghe, F.A. (1976) Transfusion malaria in Sri Lanka. *Tropical Doctor*, **6**, 4-6.

Wijesundera, M.de S., Ranaweera, R.L.A.R., Voller, A. and Bidwell,D.E.(1996) Evaluation of a malaria antibody ELISA as a malaria screening tests for blood donors. Eds. Gunawardana, R.A.L.H., Mendis, B.R.R.N. and Careem, M.A. Proceedings of the Annual Research Sessions 1996, University of Peradeniya. pp.115-118.