MALARIA SCREENING OF BLOOD DONORS IN SAUDI ARABIA

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Background: Transfusion-associated malaria is a potentially serious complication that continues to pose risks in blood bank settings. There is a need for effective malaria screening of blood donations to improve on the current exclusion policies of potentially infected carriers on the basis of clinical and travel history. We evaluated the potential usefulness of ELISA screening for malaria antibody and *P. falciparum* antigen among Saudi blood donors.

Materials and Methods: A total of 1756 donors were studied, 1100 from the malaria endemic Southern Region and 656 donors from the known malaria-free Riyadh area. Results: The overall antibody prevalence for the antibody was 7.6%, in comparison to only 0.17% for the antigen. In the endemic region, the antibody positivity rate of 9.1% was almost double the rate in the non-endemic area (4.8%). There was no difference in the antigen prevalence rates; 0.18% in endemic and 0.15% in non-endemic areas.

Conclusions: In malaria endemic countries like Saudi Arabia, excluding antibody-positive donations would result in too much wastage of blood units. However, antigen malaria testing appears to offer a potential utility, as only a few donations would be rejected.

Golden malaria due to *Plasmodium falciparum* can be acquired even with transfusion of a small number of infected red cells. Such infection can be life threatening in a patient population which is already poorly and because of possible delay in diagnosis, being unexpected. In Saudi Arabia, the exact incidence of transfusion malaria has not been established. However, the existence of a significant risk has been highlighted in a recent report that documented 12 cases of *P. falciparum* transfusion-associated malaria in one center in Riyadh over a period of four years, and also quoted another 20 unreported cases from other centers in the Kingdom. Furthermore, two cases of postoperative transfusion malaria following cardiac surgery were reported from Madina and another two neonates transfused from the same blood donor were described in Riyadh. These documented cases are likely to be an underestimate of overall burden of transfusion malaria that can only be measured through a comprehensive national surveillance program.

Laboratory screening for malaria remains a possible option for reducing transfusion malaria. Traditional blood film microscopy involving large numbers of blood donor samples is labor-intensive and requires high technical skill for accurate diagnosis of malaria, particularly if the parasitaemia is low. Moreover, its sensitivity decreases in parallel with the density of malaria parasites in the blood. Immunological and molecular techniques can be used alternatively. There are now commercially available enzyme-linked immunosorbent assay (ELISA) kits for detecting malaria antigen and antibody. The kits can detect antigen and antibody reliably and can be quite specific. The utility of antibody kits have been evaluated in the United Kingdom for screening selected donors who are at risk of malaria.

The antibody-based tests were found to be useful for releasing an estimated 40,000 units of blood annually. In this study, we assessed the performance of both malaria antigen and antibody ELISA in: 1) Saudi blood donor population from the endemic Southern Region and the non-endemic Central Region, and 2) in a clinical setting of
Prevalence of *P. falciparum* antigen and malaria antibody among Saudi blood donors from endemic (Khamis Mushayt) and non-endemic (Riyadh) areas.

<table>
<thead>
<tr>
<th>Area</th>
<th>Number tested</th>
<th>Antigen</th>
<th>Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Khamis Mushayt</td>
<td>1100</td>
<td>2 (0.18%)</td>
<td>101 (9.1%)</td>
</tr>
<tr>
<td>Riyadh</td>
<td>656</td>
<td>1 (0.15%)</td>
<td>32 (4.8%)</td>
</tr>
<tr>
<td>Total</td>
<td>1756</td>
<td>3* (0.17%)</td>
<td>133** (7.6%)</td>
</tr>
</tbody>
</table>

*Samples were negative by blood film microscopy and PCR-negative; **seven randomly selected samples tested PCR-negative.

Sequential malaria results in a symptomatic 6-years old child.

<table>
<thead>
<tr>
<th>Time</th>
<th>Microscopy</th>
<th>ELISA antigen</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Admission</td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>After 16 hours</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>After 24 hours</td>
<td>Positive</td>
<td>Positive</td>
<td>Not done</td>
</tr>
</tbody>
</table>

Materials and Methods

Blood samples from 1756 blood donors derived from two geographically different centers in Saudi Arabia were used in study. Of these, 1100 samples were obtained from blood donors attending the Armed Forces Hospital, Khamis Mushayt in the Southern Region. The remaining 656 samples were from the Armed Forces Hospital Blood Bank, Riyadh. All samples were procured through voluntary participation of donors for the routine blood donor screening. The specimens were collected from consecutive donors during a three-month period from January to March 2000, a period coinciding with maximum malaria transmission in the endemic area. In addition, serial bleeds were also obtained from a six-year-old child with the onset of a febrile illness that was consistent with malaria, and from three adults following documented *P. falciparum* infection. Plasma was separated from each specimen and frozen at –20°C until tested. Stained blood films were prepared for blood donors for microscopy.

Immunological Assays for Malaria Antibody and Malaria Antigen

Malaria-specific antibody and antigen were detected by using ELISA kits (Malaria Antibody CELISA and Malaria Antigen CELISA, Cellabs Pty Ltd, Brookvale NSW, Australia). In brief, antibodies present in the blood samples were detected by reacting the samples in wells coated with malaria antigen prepared by sonication of *P. falciparum*-infected red blood cells. Specific binding was quantified by using a monoclonal anti-human IgG to capture the *P. falciparum* type 2-specific histidine-rich protein (HRP-2). In the antigen test, HRP-2 was quantified by a capture ELISA technique by using monoclonal antibodies for capture as well as for detection purpose. An enzyme chromogen, tetramethylene benzidine dihydrochloride (TMB), was used which upon adding release the color the intensity of which is reciprocal to the amount of the antibody present in the sample. The antigen test was specific to *P. falciparum*, whereas the antibody test is known to react with all four species (*P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*).

**Molecular Analyses of Positive Blood Samples**

All specimens positive for malaria antigen and seven randomly selected samples positive for malaria antibodies were tested for presence of malaria DNA by using the polymerase chain reaction (PCR) assays available through the Malaria Reference Laboratories located in United Kingdom (Department of Clinical Parasitology, Hospital for Tropical Diseases, London) and South Africa (Department of Molecular Medicine and Hematology, Wits Medical School, University of Natal).

Results

A total of 1756 blood samples were tested for the presence of malaria-specific antibodies and antigens and results are shown in Table 1. The overall seropositive rate for malaria antibody was 7.6%, whereas the antigen was detectable among only 0.17% of the screened donor population. Out of 1100 samples from the endemic area tested, 101 (9.1%) samples were positive for antibody whereas only 2 (0.18%) samples were positive for antigen test. Out of 656 samples from non-endemic area tested, 32 samples (4.8%) were positive for antibody whereas only one sample was positive for antigen. The antibody prevalence in the endemic Khamis Mushayt area (9.1%) was approximately double the rate found in the non-endemic Riyadh region (4.8%). This is a reflection of the higher malaria endemicity in the South and also the mobility of exposed donors from endemic areas to Riyadh which is recognized as a malaria-free part of the country. The blood films from donors with either malaria antigen or antibody reactivity were examined microscopically and were all negative.

Table 2 shows the various malaria results of the six-year-old child. Negative blood film microscopy on admission was verified in three laboratories while *P. falciparum* trophozoites were easily demonstrated in the subsequent blood samples. By contrast, both malaria antigen and PCR tests were positive in the first specimen, thus indicating the higher sensitivity of these assays. Serial blood specimens from the three adults who recovered from acute *P. falciparum* infections demonstrated that the malaria antibody remained detectable for about six months in two patients who were renal transplants, and for more than two years in the third patient (Figure 1). The
latter patient never left Riyadh during the period of this follow-up.

Discussion

The overall malaria antibody prevalence of 7.6% is a reflection of malaria endemicity in this country. By contrast, a 0.1% prevalence rate has been shown among UK donors where this malaria antibody has been found to be effective in the screening of selected at-risk donors.7 In our blood bank, discarding such a high number of reactive donations will be difficult as it will result in blood shortages when most of these donors probably had recovered completely from what is essentially an acute P. falciparum infection, the most common type of malaria in the Saudi Arabia. Further complications will be the donors counseling, management and their subsequent deferral period. The latter might extend to several years as antibody persistence has been clearly demonstrated by the follow-up of one of our patients. Thus, great care is necessary in devising appropriate testing methods to overcome any loss of donors.

In contrast to the antibody assay, the P. falciparum antigen test will allow for discarding a very small number of units estimated at about 0.2% in this study. In the symptomatic patient, the early positivity of the antigen test that was confirmed by PCR, demonstrated a higher sensitivity over traditional microscopy. This finding encourages the use of the antigen test as a donor screening procedure. However, the asymptomatic blood donor could pose a more subtle challenge as suggested by the three antigen-positive but PCR-negative donations. HRP-2 positivity has rarely been reported in unexposed persons,8 therefore, these three cases may be due to the persistence of antigenemia that has been shown to remain in the blood for upwards of 28 days after cure of P. falciparum infection.9,10 Unfortunately, it was not possible to recall these donors for a serological follow-up and clinical evaluation that might have further clarified the exact significance of these findings.

Several studies have demonstrated an overall high sensitivity of HRP-2-based diagnostic assays and their potential clinical utility for the diagnosis of malaria in symptomatic patients.8,9,11 The demonstration of HRP-2 positivity in our young patient, hours before microscopy became positive for P. falciparum is consistent with these studies. In the present study, though, we subjected the antigen assay to an evaluation in apparently healthy blood donors to assess its diagnostic value in this asymptomatic population. According to the manufacturers, this antigen assay has a sensitivity of 98%, a specificity of 96% and a parasitemia detection limit of 0.001% (equivalent to 50 parasites/µl) and has been designed to supplement conventional microscopy of blood films in clinical settings.

In the blood bank setting, time is crucial as demand for blood components is usually urgent, requiring that screening procedures be rapid and accurate. These logistics requirements push the operative procedures to the large scale and therefore immunological techniques are favored. The antigen test has a turnaround time of approximately three hours and uses proven microwell plate immunoassay techniques that can be run with a minimal amount of equipment.

Minimizing the risk of transfusion-induced malaria involves deferral of donors with a positive history of recent clinical malaria attack. This strategy is undertaken in Saudi Arabia and in accordance with the Ministry of Health directives for prospective donors to specifically answer this point. However, deferral policies designed to exclude potentially infected donors on the basis of history of travel to or residence in a malarious area is not practical in endemic areas because continuous exposure means the exclusion policy would disqualify nearly all donors. It can also be difficult to obtain accurate travel and immigration histories.12 Furthermore, clerical errors may occur.13 Another limitation to current exclusion guidelines is the occasional prolonged, asymptomatic persistence of malaria parasites in some infected persons that may result in some cases of transfusion malaria. Despite the progress made in malaria control, malaria transmission is still endemic in the South and Southwestern parts of the country where P. falciparum accounts for over 90% of cases.14 Inevitably, a pool of potentially infectious donors will be established among some asymptomatic carriers in that region. In malaria-endemic areas, it is known that acquired immunity against the disease is associated with low-grade parasitemia without symptoms and is a common phenomenon in adults.15 Without an effective method for screening blood donors, this ever-present risk of transmitting malaria by transfusion is further increased by a significant expansion of international travel to other malarious countries, and through the voluntary contribution of blood donations by expatriates from various endemic areas. Malaria epidemics like the outbreak of 1998/1999 season may confound the problem even further.

We are planning to expand this preliminary study to show whether this P. falciparum antigen assay may be sufficiently sensitive and specific to detect low-level parasitemia. This additional data is needed to help determine the most appropriate and cost-effective screening tests available, and most importantly, to suggest a workable national malaria screening policy that should give an improved level of blood safety to what is currently in place.

References


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