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Original Article

RISK OF MALARIA TRANSMISSION THROUGH BLOOD TRANSFUSION AND ITS DETECTION BY SEROLOGICAL METHOD

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ABSTRACT:

Objective: To assess the risk of transmission of malaria through blood transfusion, and compare efficacy of testing by immunochromatographic(ICT) devices vis a vis peripheral blood film (PBF).

Design: 300 blood samples were tested divided into three equal groups of healthy volunteers, voluntary non-remunerated blood donors and patients suffering from malaria. Testing was carried out by a serological screening method, together with observation of peripheral blood films.

Setting: Samples were collected from different sites and tested at the Institute of Haematology & Blood Transfusion Service, Punjab.

Subjects: One hundred blood donors were selected from persons donating blood at the Institute or on mobile sessions. An equal number of healthy controls were students and staff of different colleges & the Institute. Samples of 100 patients of pyrexia and diagnosed clinically as suffering from malaria were collected from multiple clinics, laboratories and hospitals in Lahore.

Main outcome measures: Assessment of the risk of transmission of malaria through blood and blood products & the comparison of serological testing for malaria with conventional peripheral blood film detection.

Results: Amongst healthy blood donors we did not find even a single case of malaria and there was no report of persistent post transfusion pyrexia. We are unable to comment on species frequency in blood donors. However, amongst known patients of malaria we found a higher frequency of Plasmodium vivax(P.v) as compared to Plasmodium falciparum(P.f). Testing by serological method, helped us to diagnose 5% of our patients who were missed by peripheral blood films.

Conclusion: Between properly selected voluntary non-remunerated blood donors the incidence of malaria transmission is zero and the blood is safe for transfusion. Serological testing shows good correlation with peripheral blood film detection. In fact, it can detect the disease even when film detection has been unsuccessful. If proper donor selection criteria are observed there is little risk of transmitting malaria through transfusion. However, as the donor pool in the Service is not necessarily totally that of voluntary non-remunerated donors and substantive numbers of replacement/first time, occasionally uneducated/unaware donors, are being bled, screening for malaria will not be totally unrewarding.

KEY WORDS: Blood safety; risk of malaria transmission; efficacy of serological testing.

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INTRODUCTION

Malaria is an acute and chronic disease caused by obligate intracellular protozoa of the genus Plasmodium with four recognized species. Transmission to humans occurs by a vector, the female Anopheles mosquito. The illness that follows is highly variable but is usually characterized by paroxysm of fever, chills, anaemia and splenomegaly. The disease occurs worldwide with an estimated 2 billion inhab-

itants at risk. Globally, there are well recognised endemic areas and Pakistan is situated in the malaria endemic zone where natural transmission can easily occur[1].

Semi-immune individuals are capable of sustaining asymptomatic parasitemias and it is difficult to ascertain the precise relationship between symptoms and parasitemias. Individuals have been known to harbour the parasite and remain asymptomatic for years[1]. Plasmodium falciparum is absent from the peripheral blood for a portion of its life cycle and because even symptomatic parasitemias are sometimes below the limits of microscopic detection, it is possible for individuals to be aparasitemic and to have malarial illness. Furthermore, a single negative blood film test cannot necessarily exclude the diagnosis[1].

Rarely, direct inoculation of infected red blood cells i.e, transfusion malaria (TxM) or malaria from contaminated needles as a result of needle sharing amongst intravenous substance abusers have been reported[1]. The first case of transfusional malaria(TxM) was reported in 1911 and since then 3000 cases worldwide have been described. The incidence of TxM ranges from as low as 0.25 cases per million units transfused in the US and UK to more than 50 cases per million in some endemic areas[2].

Increased susceptibility may be seen as a result of increased international travel and immigration. Migration of asymptomatic carriers who might be potential blood donors in non endemic countries calls for meticulous history taking from prospective blood donors. In endemic areas, transmission from symptomatic donors is unlikely because such individuals are usually excluded as donors on interview. TxM is prevented, by applying a policy of donor deferral for variable periods as follows[2]:

1. Permanent residents of non-endemic countries who travel to endemic areas are deferred for one year.
2. Prospective donors who have had malaria are advised to donate after a three year asymptomatic period spent outside an endemic area. (In Pakistan, the recommended deferral period is six months[3]).
3. Immigrants, refugees, citizens or residents of endemic countries are deferred for 3 years.

When one or more deferrals prevail, it is wise to apply the longest period. As individuals may be parasitemic for many years, and yet remain asymtomatic, careful history taking is pertinent in identifying prospective donors at risk for transmitting malaria.

Malarial parasites survive for at least one week at 4°C in whole blood and red cell concentrates and for the same period of time in platelets at room temperature[4]. A policy of holding up to two weeks, to achieve a relative protection from TxM, is occasionally employed. Donations used for preparing plasma, plasma components or derivatives devoid of red blood cells are excluded from the application of this policy; however malaria following transfusion of cryoprecipitate has been reported[5].

A wide variety of laboratory methods have been developed to establish a diagnosis; these include flow cytometry, fluorescent microscopy, centrifugal diagnosis, immunodiagnosis including ICT and EIA as well as nucleic acid probes like PCR[2]. Currently, none are being used routinely. The choice of a screening test for malaria is difficult, although detection of the parasite on thick or thin peripheral film is considered the gold standard, there are limits on the parasitologic diagnosis by this method due to the fact that Plasmodium falciparum is absent from the blood during a portion of its life cycle. Further blood films are a cumbersome and time-consuming method that requires expertise and industry on the part of the laboratorian, which is overtly lacking in our scenario. This is compounded by the blind therapy for fever usually given to our patients. Consequently, most peripheral blood films for malarial parasites are reported to be negative. Alternative screening or diagnostic methods that have been developed to cope with these deficiencies should be employed especially in malaria endemic areas.

One such method evaluated in this study is the antigen detection technique. This is a

simple dipstick antigen capture assay based on qualitative detection of Plasmodium falciparum histidine rich protein-2(Pf HPR-2). The technique is feasible in the field and has been reported to show good sensitivity and specificity[1]. This method needs little equipment & training, and is said to become negative only after a week of anti-malarial therapy. A joint Plasmodium falciparum & vivax dipstick is also available which was employed in this study. Both whole blood and serum can be used. Development of dipsticks for other plasmodia species is in the process.

This study estimates the frequency of malaria in voluntary non-remunerated blood donors and assesses the subsequent risk of developing TxM. Secondly, it attempts to evaluate the dipstick method as a screening test for malaria and compares it with the generally accepted gold standard of detection by the peripheral blood film.

MATERIALS AND METHODS

Study Population:

Three hundred blood samples were collected from three equal groups as follows:

Group-I: Healthy volunteers as negative controls; these were selected from students & staff of the Institute and of other colleges.

Group-II: Voluntary non-remunerated blood donors as test subjects from persons donating blood at the Institute or on mobile sessions. Donors with history of malaria or those having taken treatment during the last six months were excluded as per National Guidelines.

Group-III: Samples of patients of pyrexia and diagnosed clinically as suffering from malaria were collected from multiple clinics, laboratories and hospitals.
All subjects were aged between 18-60 years; sex distribution was ignored.
All transfused units were followed up for post transfusion pyrexia persisting beyond one week.

Sample Collection:

Blood samples were collected as follows:

1. 2.0 ml clotted blood
2. Two freshly prepared slides of peripheral blood (one thick & one thin).

Serum obtained by centrifugation at 3000 rpm for 10 minutes was used for serological testing. The peripheral blood films were stained by Giemsa, the thick film without fixation.

Testing algorithm:

1. 10ul of the serum was placed in the test well of the device followed by 3 drops of assay diluent. Results were read after 20 minutes at room temperature. The appearance of a colour band in the result window at the delineated control slots for P.f/P.v depicted the proper working of the device. Test positivity was shown by appearance of colour band at the test slot.
2. Two experienced observers evaluated the peripheral blood films. Species identification was done on the thin film.

RESULTS

Table-I shows the results of malarial screening in the three groups studied by serology including species identification in the patient population by the same methodology.

Table-II shows the result of malarial screening in the study population by microscopy of peripheral blood films by two experienced observers.

Table-III compares the diagnosis and species identification in the patient population by both methods.

There were no reports of post transfusion

Table-I: Frequency & Species distribution in the study population by serological method

Study Groups n=300	*Plasmodium Falciparum*	*Plasmodium Vivax*
Healthy Controls n=100	Nil	Nil
Blood Donors n=100	Nil	Nil
Patient population n=100	31	69

pyrexia, persisting beyond one week, for any of the units transfused.

Table II: Frequency & Species distribution in the study population by evaluation of peripheral blood films

Study Groups n=300	*Plasmodium Falciparum*	*Plasmodium Vivax*	*Negative PBF*
Healthy Controls n=100	Nil	Nil	All
Blood Donors n=100	Nil	Nil	All
Patient population n=100	28	67	05

Table III: Comparison of diagnosis & species identification between serological testing & peripheral blood films

Methodology	*Species Identification*			
	P.Falciparum		*P.Vivax*	
	Positive	*Negative*	*Positive*	*Negative*
PBF	28	03	67	02
Serological test	31	00	69	00

DISCUSSION

As Pakistan is situated in the malaria endemic zone, transmission of this disease through blood transfusion is a serious public health concern. Public sector blood banks, at present, do not routinely screen for malaria. Reliance is placed on pro-active approach of appropriate donor selection and deferral criteria. Unfortunately, blind therapy for pyrexia/malaria is a common practice in our local scenario. This, at times, complicates laboratory diagnosis.

There have been no reported cases of TxM in Pakistan. Whether this is the true situation or an ignorant scenario, stimulated us to evaluate our blood donors for assessment of risk of transmission as well as to determine a user-friendly method for screening.

We found a 0% transmission risk in a well-selected donor population. Generally, TxM is rare; no case of TxM has been reported in France since 1994 after requirement of notification of possible cases by Health authorities was introduced[6]. In the US, the incidence is 0.25 cases per million units transfused or 1-3 new cases/year[7]. In Canada, it is considered to be rarer with only three reported cases in medical literature[8]. In New Zealand, where deferral is currently practiced, it was estimated that only 1.7% of those deferred were positive for malarial antibodies and if screening is introduced it could lead to recovery of 2300 units of red cells per year[9].

It is generally believed, that careful screening of donors according to recommended exclusion guidelines remains the best way to prevent transfusion-transmitted malaria. It is also held that malarial screening should follow clinical and epidemiological criteria suitable to each region[10]. The criteria of exclusion or deferral of donors is variable throughout the world. In Europe donors are excluded from donations for six months with or without testing for malaria after return from endemic area[6]. Donors in Europe are excluded for 5 years on history of malaria if they test positive for anti-malarial antibody; in the US, UK and Denmark it is however 1year, in Ireland it is 3 years without antibody testing[11]. Donors working at airports where flights land from endemic areas are considered as an occupational hazard group in some countries[6]. Our deferral policy of exclusion for six months[3] is considerably lax as compared to international standards. The duration of transmission of infection by different species in asymptomatic parasitemia is variable. P.falciparum is said to be eliminated within 2 years, P.vivax and P. ovale after 3 years, while P malariae may persist even longer[12]. Further, in view of lack of routine screening for malaria in our country and deficiencies in donor selection process, the prevailing deferral policy needs rethinking.

As we could not detect even a single case of malaria in our blood donors we cannot comment on species frequency. However, by and

large, P.falciparum is the most dreaded species in TxM. Amongst patients, we found a higher frequency of P.vivax as compared to P.falciparum; this is contrary to the findings of some others[13]. Further, detection of P.falciparum being higher by serological testing than merely by blood smears is explainable on the basis of the exo-erythrocytic cycle of this species. The two cases of P.vivax which skipped detection by PBF could have been due to blind treatment they may have received.

Our findings regarding ICT as a screening methodology is favourable. It needs little staff training and expertise and was able to detect 5 patients who were negative by PBF. Our sample size is small and further evaluation will be needed to address the issue of its sensitivity and specificity. Testing for anti-plasmodial antibodies have been employed by a number of workers and found to be satisfactory[12], although some species cross reactivity have been noted. Some have even employed PCR detection. This method is considered to be sensitive even if a minimum of 5 malarial parasites are present in the blood, while a parasite count of 5×10^3 /ml is needed for satisfactory positive detection by thick smear[6].

The universally employed test of PBF for malaria detection can at times fail to diagnose the disease. It is therefore advisable to test all PBF negative, clinically suspicious cases of malaria by a second method such as immunochromatographic (ICT) devices. From the public health perspective, this is important as a failure to pick up cases may lead to a surge in the incidence of pyrexia of unknown origin. This could perforce result in over-investigation and treatment that our patients can ill afford. A simpler testing method such as by ICT devices would make a lot of difference in our rural scenario as it requires little expertise and can be undertaken at any clinic or dispensary.

CONCLUSIONS

1. Blood donated by healthy well-selected biood donors bears little risk of transmission of malaria.
2. The present National guidelines regarding donor deferral for malaria needs rethinking.
3. Although peripheral blood film is the gold standard for the diagnosis of malaria, testing by ICT devices can be considered for initial donor screening.
4. As transmission from asymptomatic carriers in an endemic area is a possibility, donor screening for malaria in Pakistan is called for to fortify blood safety.

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