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Malaria Screening among Blood Donors in Non Endemic Malarial Regions: Role of Donor History Questionnaires and Rapid Malaria Diagnostic Test

Doaa Abdelbadie Salem[1], Azza A Baiomy[2,*]

[1]Parasitology, Mansoura Faculty of Medicine, Mansoura University, Egypt
[2]Clinical Pathology, Mansoura Faculty of Medicine, Mansoura University, Egypt
*Corresponding author: azzabaiomy263@yahoo.com

Abstract Malaria is one of the most important parasitic diseases responsible for some cases of transfusion transmitted disease in the world. Malaria screening test of blood donor may increase the safety of blood donations. In response to the information about a *Plasmodium vivax* malaria outbreak in the Aswan Governorate in Egypt 2014 and in the absence of a licensed test for donor screening, our aim was to assess the value of malaria donor history questionnaires and rapid malaria diagnostic test in malaria screening among blood donors to increase the safety of blood donation. Out of two hundred and fifty two healthy blood donors attend the Mansoura University blood bank unit for blood donation, between July to August, 2015, twelve donors were deferred, one from a malaria endemic area and one with a history of travel to a malaria endemic area, the rest was deferred for other reasons. All donors were checked for weight, pulse rate, blood pressure, temperature, hemoglobin levels and malaria donor history questionnaires. Screening for malaria was done by Microscopic Examination of Thick Blood Films stained with diluted Giemsa stain and by using the individual rapid malaria test OptiMAL-IT (BIO-RAD, France) for all blood donor samples. No positive samples of blood donor volunteers were detected neither by microscopic examination, nor by rapid malaria test OptiMAL-IT. Our study support that, in a non endemic malaria region, donor deferral by malaria donor history questionnaires and screening of blood donors for malaria by using thick blood films or by rapid malaria test OptiMAL-IT is enough for safe blood donation, but clear guidelines are mandatory.

Keywords: *transfusion-transmitted malaria, enzyme immunoassay, donor's risk*

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1. Introduction

The transmission of parasitic organisms through transfusion is relatively rare, in comparison to that of bacterial and viral infections. The most common parasitic organisms implicated in transfusion-transmitted infections, Plasmodium spp., Trypanosoma cruzi, Babesia microti, Toxoplasma gondii, Leishmania spp. Etc, the major parasitic transfusion-transmitted diseases is malaria in tropical countries [1].

Malaria is a protozoan infection of red blood cells usually transmitted by the bite of a blood feeding female anopheline mosquito, but cases of transfusion-transmitted malaria (TTM) have been recorded in the United States [2], Switzerland [3], France [4] and the United Kingdom [5].

It was reported that the four principal species of Plasmodium can be acquired through blood components such as red cell concentrates, platelets [6], leucocytes [7], and frozen red blood cells [8].

Malaria infection has become of more interest to blood banking and blood transfusion based on discoveries that malaria infection may cause methaemoglobinaemia, as haemoglobin taken up by the parasites into their acid food vacuole leads to the spontaneous oxidation of ferrous (Fe^{2+}) to ferric (Fe^{3+}) iron. Methaemoglobin is a dysfunctional form of hemoglobin that is incapable of transporting oxygen [9]. In addition, the remaining monomers of ferrous haem within a haemoglobin tetramer bind their oxygen more tightly causing a left shift of the oxygen dissociation curve and reduced oxygen delivery at the tissue level. When Methaemoglobin concentrations are increased, there is less available functional haemoglobin to carry oxygen for systemic delivery leading to varying complications from skin discolouration, cyanosis, weakness, confusion seizures and death. [10]

Blood donor testing for malaria parasitaemia is not routinely done in the transfusion laboratory in Egypt. This could mean that a relatively high probability of malaria transmission through blood transfusion of asymptomatic donor units.

2. Subjects and Methods

2.1. Subjects

Our study conducted on two hundred and forty (240) random healthy blood donors aged 18–45 years attend the

blood bank unit of Mansoura university hospital for blood donation purposes between July to August 2015. Blood donor volunteers with age less than 18 years and more than 60 years, history of medical problem especially hypertension, jaundice, anemia, bleeding disorders, hospitalization at fever hospitals, underweight (Body weight less than 50 Kg), pregnancy or recent delivery less than 12 weeks and presence of any malaria deferral criteria were deferred from this study. Informed consents were taken from everyone enrolled in this study.

2.1.1. Malaria Donor Deferral Criteria (according to FDA, 2013) [11]

1. A donor who has a history of malaria is deferred for 3 years and if that donor has remained free of malaria symptoms for a 3-year period while residing in a non-endemic country, the Medical Director may decide to accept the donor, provided the donor meets all other donor eligibility criteria.
2. Defer a donor who had been a prior resident in a malaria-endemic country for 3 years. After the 3-year deferral period, the donor may be eligible to donate provided the donor has been free from malaria during this period and meets all other donor eligibility criteria.
3. A donor who travels to a Malaria-endemic Area is deferred for 1 year after the last departure from a malaria-endemic area, a donor who is a resident of a non-endemic country and who has traveled to or through any malaria-endemic area, whether or not the donor has received malaria chemoprophylaxis. After the 1-year deferral period, the donor may be eligible to donate, provided the donor has been free from malaria during this period and meets all other donor eligibility criteria.
4. Defer for 3 years after a visit to a malaria-endemic area a donor who is a prior resident of a malaria-endemic country and who has been a resident of non-endemic countries for less than 3 consecutive years. After the 3-year deferral period, the donor may be eligible to donate, provided the donor has been free from malaria during this period and meets all other donor eligibility criteria.
5. A residence for 3 years consecutively in non-endemic countries, that you defer that donor for 1 year from the time that they return to the non-endemic country. After the 1-year deferral period, the donor may be eligible to donate, provided the donor has been free from malaria during this period and meets all other donor eligibility criteria.

All donors were subjected to donor history questionnaires, weight, pulse rate, blood pressure, temperature, hemoglobin levels and screening for malaria by both thick blood film and OptiMAL test.

2.1.2. Malaria Donor History Questionnaires

1. A history of prior residence in a malaria-endemic country
2. A history of malaria in the past 3 years;
3. A history of treatment with anti-malarial drugs;
4. A history of travel to a malaria endemic area in the past one year with or without receiving anti malarial prophylaxis.
5. A history of travel to a malaria-endemic area in the past three years, if previously a resident of a malaria-endemic country.

2.2. Methods

2.2.1. Preparation of Thick Blood Film and Microscopic Examination:

A drop of blood was placed middle of a clean microscope slide and with the corner of a second slide spread the drop until to make an area of approximately 1 cm^2 . It should just be possible to read small print through a thick film. Then the film is air dried and NOT fixed in methanol. The smear was stained with 10% Giemsa for 10 minutes and analyzed by light microscopy and examined microscopically for the presence of circulating malaria parasites using a 100× objective. A minimum of 200 consecutive fields was counted in the thick blood film before classifying a slide as negative [12].

2.2.2. OptiMAL Test:

Rapid malaria test OptiMAL-IT (BIO-RAD, France) for all samples was done according to manufacturer instructions. Optimal IT test is an immune-chromatographic test which uses monoclonal antibodies against Plasmodium-specific pLDH enzyme. These monoclonal antibodies are lined into two lines in the test, one specific for Plasmodium falciparum and the other for pan-specific monoclonal antibodies which react with all four malaria species which infect human (*P. falciarum, P.vivax, P. ovale* and *P. malariae*). Interpretation of the test results was done.

Briefly, we wrote the name and the date of the test on the cassette then we added one drop of buffer in well one and four drops of buffer in well 2 then leave to stand for one minute then we added 10 µL of blood in well 1. Then by using the flat end of the dropper stir we mixed the blood with buffer in well 1 for one minute to disrupts the red blood cells and releases the pLDH. Then we left the test cassette in well 1 vertically for 10 minutes after that we removed the test cassette and it was placed in well 2 and was left to stand for 10 minutes to clears the hemoglobin from the strip.

2.2.3. Interpretation of Results:

- A test result was considered valid if the control line was visible.
- A Positive test for *P. falciparum* (± *P. vivax, P. oval, P. malariae)*: one control band and two test bands.
- A Positive test for *P. vivax / P. oval/ P. malariae*: one control band and one test band.
- A Negative test: One control band at the top of the strip.

Data were analyzed using numbers and percentage for qualitative variables.

3. Results

Demographic data of the studied subjects showed that 218 blood donors were male and 22 of them were female.

The median age of the blood donors was 22 years. Donors between age 18 and 30 years constituted the largest portion (47%) Table 1.

Table 1. Demographic data of studied blood donors

Variable	Frequency	
	Number	Percent
Gender		
Male	218	91%
Female	22	9 %
Age group		
18-30 years	113	47%
31-40 years	82	34.2%
41-45 years	45	18.8%

All donors showed normal blood pressure, pulse rate, hemoglobin level, and temperature and were qualified by the questionnaire. No positive samples of blood donor volunteers for malaria were detected neither by microscopic examination, nor by rapid malaria test OptiMAL-IT.

By checking the blood donors by donor history questionnaires, twelve of them were deferred from this study, one of them from a malaria endemic area and other with a history of travel to a malaria endemic area, the rest were deferred for other reasons Table 2.

Table 2. Deferred cases according to deferral criteria

Variable	Number
Malaria deferral criteria	
- A history of prior residence in a malaria-endemic country	1
- A history of malaria in the past 3 years	0
- A history of treatment with anti-malarial drugs	0
- A history of travel to a malaria endemic area in the past one year with or without receiving anti malarial prophylaxis.	1
- A history of travel to a malaria-endemic area in the past three years, if previously a resident of a malaria-endemic country.	0
Body weight less than 50 Kg	1
History of prior hospitalization at fever hospitals	3
History of liver disease	3
Age less than 18 years	3

4. Discussion:

Transfusion transmitted malaria (TTM) especially in malaria endemic countries can be a major problem because those who are semi-immune individuals with low level of parasitemia remain asymptomatic and may be qualified as blood donors.

TTM is very rare in countries in which malaria is not endemic. Malaria is currently re-emerging in many areas where it had been eradicated in the past, malaria outbreak in Aswan Governorate in Egypt, were reported in late May 2014, 19 locally acquired cases have been confirmed according to information received by federal health officials [13]. Mansoura city where the study was conducted had a lot of students come from endemic areas e.g Sudan and Saudi Arabia.

Our aim was to assess the importance of malaria donor history questionnaires and malaria screening among blood donors to increase the safety of blood donation.

Examination of thick blood smears is not cost-effective for screening large numbers of donors, nor is it sensitive enough (limit of detection is approximately 50 parasites/μL) to detect low levels of parasitemia that might exist in donors. But this method remains the gold standard for the diagnosis of malaria [14].

OptiMAL is a malarial antigen capture assay which detects Plasmodium-specific pLDH enzyme, Anti-pLDH monoclonal antibodies used in the test present only in live parasites leading to high sensitivity better than 90%. It also allow the differentiation between Plasmodium falciparum and non-Plasmodium falciparum infections where one specific for P. falciparum and the other two are pan-specific for all. However, its limit is about 100 parasites/μL and it can not detect mixed infections. It offer the possibility of more rapid non microscopic method, thereby saving on training and time (15-20 minutes) [15].

Polymerase chain reaction (PCR) methods to detect plasmodium DNA or RNA may be the most sensitive (five parasite/ μL), especially in the case of mixed infection and low parasitaemia and specific but are required high expertise, expensive infrastructure and its consumables and reagents are with high cost [16].

Because of Egypt's constrain economy which limit the implementation of PCR in screening of malaria in blood bank we suggest usage of proper malaria donor history questionnaires with thick blood film and OPTIMAL IT for malaria screening in blood bank.

One study, however, has indicated even this method may not be able to detect organisms below the level of 10 parasites/10 μL blood [17]. Detection of mixed infection which is done by PCR had no advantage in blood bank.

Saad M. Bin Dajem et al., (2014) [18] who conducted a study in Aseer region (Southern part of KSA) where many cases of malaria were reported. They found that all samples of blood donor volunteers were negative by peripheral blood smear, rapid diagnostic test and by PCR. They recommended OptiMAL IT rapid diagnostic test to replace the peripheral blood smear method in blood banks.

5. Conclusion

Our study support that, in non endemic malaria regions, donor deferral by malaria donor history questionnaires and screening of blood donors for malaria by using thick blood films and by rapid malaria test OptiMAL is enough for safe blood donation, but clear guidelines are mandatory.

Statement of Competing Interests

The authors have no competing interests.

Recommendations

We advocate for standard donor screening policy for malaria with accurate identification of donors with the potential to transmit malaria through use of a malaria donor history questionnaires and screen for malaria with

thick blood films and by rapid malaria test OptiMAL is enough in non endemic areas.

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