Comparative evaluation of bivalent malaria rapid diagnostic tests versus traditional microscopy method in assessment of malaria in blood donors at a tertiary care teaching hospital and regional blood transfusion centre in central India

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Abstract

Background: Global malaria control efforts are based on 2 broad components: vector control and improved diagnosis and treatment of patients with clinical malaria. Until recently, conventional diagnosis of malaria has been based on either clinical diagnosis or use of microscopic examination of peripheral blood smears. The microscopic detection of blood though considered the gold standard for malaria diagnosis, it is quite laborious and require adequate technical skill and man power. This had urged the development of other microscopic malarial and rapid detection test based on the detection of malarial parasite antigen in the blood. The purpose of this study is to evaluate results of Rapid diagnostic tests for malaria and to corroborate the results with microscopy. Material and Method: This study targeted 10,310 units of donor blood, which were screened for malaria by RDT during the period of February, 2017 to April, 2018 at Tertiary Care Teaching Hospital and Blood Transfusion Centre, R D Gardi Medical College, Ujjain. Peripheral smears were analyzed to confirm the malaria parasite. Result: Nineteen (0.18%) donors were found to be malaria antigen positive, of which only three (15.8%) were confirmed by microscopy. None of the donor had given a history of fever/malaria during pre-donation screening. Seasonal variations were observed. Conclusion: Although RDT is an important tool for malaria testing in rural settings, we suggest the diagnosis must be confirmed with microscopy method. RDTs can be an important tool for malaria testing, peripheral smear microscopy continues to be the gold standard diagnostic test for malaria diagnosis. RDT can be an important tool for malaria testing, peripheral smear microscopy continues to be the gold standard diagnostic test for malaria diagnosis.

Keywords: Blood Donors, RDTs, Peripheral microscopy

Introduction

In India, it is mandatory to test every unit of blood collected for hepatitis B, hepatitis C, HIV, syphilis and malaria [1]. If donors test positive to any of the five infections, their blood is discarded. Transmission of malaria by blood transfusion was one of the first recorded incidents of transfusion transmitted infection [2].

The frequency of transfusion-transmitted malaria varies from 0.2 cases per million in nonendemic countries to 50 or more cases per million in endemic areas [3].

Importance of recognizing transfusion-transmitted malaria lies in the fact that it can lead to febrile transfusion reaction which can falsely simulate a hemolytic transfusion reaction. It can lead to the wide spread dissemination and spread of drug-resistant malarial parasite [4].

Blood transfusion possesses a problem because the parasites keep their infective activity for at least 14 days in blood bottles stored at 4 °C, a leading cause of TTPI. The parasites survive well in frozen blood (Kark 1982). Plasma that has been frozen or fractionated has never been known to transmit malaria. The incubation period of transfusion malaria depends on the no. and strain of
plasmodia transfused, on the host and on the use of anti-
malarial prophylaxis. The World Health Organization
has recognized the urgent need for simple and cost-
effective diagnostic tests for malaria to overcome the
deficiencies of both light microscopy and clinical
diagnosis [5]. Traditional practice for outpatients has
been to treat presumptively for malaria based on a
history of fever but, a significant proportion of those
treated may not have parasites (over 50% in many
settings) and hence waste a considerable amount of
drugs [6]. This old clinical based practice is still
relevant today especially, in infants where time spent on
getting a confirmatory laboratory diagnosis could lead
to increased fatality.

It has been shown that retinopathy, the study of changes
occurring in the retina of the eye, can give good
indication of malaria, because the color and other
aspects of retina were changed as a result of particular
disease.

Various methods for malaria diagnosis are [7]: I.
Peripheral smear examination by light microscopy, II.
Fluorescence microscopy techniques, III. QBC
technique, IV. Non-microscopic Rapid Diagnostic Tests: a) Immunochromatographic tests-detection of malaria antigen by HRP-2 and pLDH detection method, b) Immunochromatographic dipstick assays used for diagnosis – ICT Pf, Para Sight F, and V. Molecular methods: PCR, LAMP technique, Microassay.

The microscopic detection of blood though considered
the gold standard for malaria diagnosis, it is quite
laborious and requires adequate skill and man power.
This had urged the development of other microscopic
malarial and rapid diagnostic tests (RDTs) based on the
detection of malarial parasite antigen in the blood.
RDTs for malaria are based on the detection of either
histidine-rich protein 2 (HRP-2), produced only by
Plasmodium falciparum, parasite specific lactate
dehydrogenase (pLDH) produced by all four species or
plasmodium aldolase from the parasite glycolytic
pathway, also found in all species.

Up to now, there is no evidence-based guidance to
indicate which malaria screening methods are effective
for use by transfusion services. More consideration
must then be given to post transfusion malaria
especially among children under five years old in order
to increase the continuous management of childhood
illnesses and death.

There are many published studies on prevalence of
transfusion transmitted infections, this study
particularly focuses on the prevalence of malaria
obtained through immunochromatography method and
subsequent confirmation of positive cases by gold
standard microscopy technique.

Aims and Objectives

- To evaluate the prevalence of Malaria in eligible
  blood donors through immunochromatography
  technique after confirmation by microscopy.

- Study the seasonal variations in the incidence of
  malaria.

Material and Method

Place of study- Regional Blood Transfusion Bank
Centre, C R Gardi Hospital and R D Gardi Medical
College, Ujjain.

Duration of study- February, 2017-April, 2018

Type of study- Cross-sectional and observational study

Inclusion Criteria- The blood donors included in
present studywere all replacement and voluntary blood
donors. Donors were selected by taking history, clinical
examination and following donors’ selection criteria
according to the Indian FDA rules and regulations for
donor selection.

Sample Collection- Written consent was taken. Blood
was collected in blood bags containing anticoagulant-
preservative solution. Ap proximately 5 ml of donor
blood was also collected in two pilot tube (Plain and
EDTA) for blood group typing and testing of infectious
diseases.

Method- We have routinely screened all donated units
of blood for malaria using RDT, based on immuno-
chromatographic methods detecting antigens, histidine-
rich protein 2 (HRP2- _P. falciparum_), and p-lactate
dehydrogenase (pLDH- _P. vivax_). (MERISCREEN, Mfd.
by Meril Diagnostics Pvt. Ltm., one step test for
Malaria (Pf/Pv) antigens).

Thick and thin smears were made and examined for
parasitic forms for all positive cases, to corroborate the
results of RDT.

Additional data analysis was conducted to examine the
prevalence trends associated with each infection.
Statistical Method- Information regarding donor was
extracted from Donor register. Donor register is filled
for eligible donors before blood collection. Computerized compilation and coding of collected data
was done. All statistical analysis was made by using
Stata (version 12, college station, Texas, USA). For
comparing various categorical variables, we used ‘Chi-square’ test of significance, ‘Yates correction’ was used at relevant places. ‘Z-test’ of variation between two means was applied to compare various means at 5% level of significance. P-value of <0.05 was considered statistically significant.

**Result**

Total of 10,310 units of donated blood was screened for malaria with two different diagnostic methods between February, 2017 and April, 2018.

Figure A: Prevalence of Malaria antigen among blood donors by immunochromatography (RDT) method. Overall malaria antigen prevalence was estimated at 0.18% in 10,310 blood donors during February, 2017- April, 2018.

Out of 10,310 blood donors, nineteen blood donors were found to be seropositive for malaria antigen. Of these, 13 blood donors (68.4%) were positive for P. vivax antigen (Pv-pLDH), 4 blood donors (21%) were positive for P. falciparum antigen (Pf-HRP2) and 2 blood donors for both antigens (10.6%). Thus, overall malaria antigen prevalence among blood donors was estimated to be 0.18%. (Fig. A)

Prevalence of malaria among blood donors by microscopy. Overall malaria prevalence was estimated at 0.03% by microscopy method in 10,310 blood donors during February, 2017- April, 2018. Only 3 out of 10,310 blood donors were found to be positive for malaria through light microscopy in peripheral smear, which makes 0.03% of total blood donors. Microscopy showed two donors to be positive for P. vivax infection and one for mixed (P. vivax and P. falciparum both) infection while negative for individual P. falciparum infection.

**Table-1.1:** Comparison of RDT with microscopy method in diagnosis of malaria among blood donors.

| RDT       | Microscopy          | Total |
|-----------|---------------------|-------|
|           | Positive | Negative |       |
| Positive  | 03       | 16       | 19    |
| Negative  | 0        | 10291    | 10291 |
| Total     | 03       | 10307    | 10310 |

Prevalence of malaria antigen with RDT is 0.18%. On confirmation of seropositive blood with light microscopy, only 15.8% came positive for malaria parasite.

**Table-2:** Prevalence of malaria among blood donors by microscopy.

| Microscopy | Positive cases(no.) |
|------------|---------------------|
| P. vivax   | 02                  |
| P.falciparum | 00                 |
| Mixed      | 01                  |
| Total      | 03                  |

Overall malaria prevalence was estimated at 0.03% by microscopy method in 10,310 blood donors during February, 2017- April, 2018.
In 10,310 blood donors presented at Blood Bank, R D Gardi Medical College, Ujjain during February, 2017- April, 2018, showing high incidence during rainy season.

On assessment, the three-microscopy confirmed malaria positive blood donors made 15.8% of total malaria antigen reactive donors. (Table 1.1) None of the donor had given a history of fever/malaria during pre-donation screening.

Seasonal variation was observed in incidence of malaria antigen reactivity. High malaria antigen prevalence was found in rainy season i.e, July-September (57.9%) followed by summer season i.e, April-June (15.8%) and autumn i.e, October-November (21%), least during winter i.e, December-March (5.3%).

Discussion

The prevalence rate of malaria antigen in our donor study population was 0.18%, nineteen donors were tested positive among 10,310 blood donors. Similar to our study, Bahadur et al[4] in their study found malaria antigen prevalence rate of 0.03% among blood donors by immunochromatographic method. In their study, out of 11,736 units screened, three units were found positive for malarial antigen. Among these three positive samples, two were positive for P.vivax and one was found to be positive for P. falciparum. These three cases were also found to be positive by microscopy.

Hence, they concluded that the use of rapid detection devices along with peripheral smear study of positive donor is a reliable method to prevent transfusion transmitted malaria in India. Anju Dubey et al[8] in their study in northern India reported that none of their donors were found positive by either Microscopy or antigen detection RDT. However, one of the donors who were deferred with history of malaria was found positive by antigen detection RDT and negative by microscopy, which accounts for 0.09% prevalence rate by antigen detection RDT among blood donors. Therefore, they concluded that blood donor screening by Microscopy may not be an acceptable method.

Malaria antigens currently targeted by RDTs are HRP-2, parasite lactate dehydrogenase (pLDH) and plasmodium aldolase (PL-ald). Moody et al[9] demonstrated that Plasmodium species secret these proteins thus the sensitivity and specificity of RDTs are measured based on them. P. falciparum has been shown to secret lots of HRP-2 more than HRP-1 and HRP-3 whereas pLDH and PL-ald are found in other species of Plasmodium. A number of studies on RDTs have been conducted, although measures of accuracy have varied widely, as a result of differences in methodology, study site epidemiology and type of RDT used i.e. histidine rich protein - 2 (HRP-2) and plasmodium specific lactate dehydrogenase (pLDH) and species specific pLDH or aldolase-based test. [10-12].

A study at New Delhi evaluated the usefulness of new rapid diagnostic test (HRP2/ pLDH Malaria card test) for malaria diagnosis in the forested belt of central India. Their analysis revealed that in comparison to microscopy RDT was 93% sensitive, 85% specific with a positive predictive value of 79% and a Negative predictive value of 95%. (13)

Jessica Martha et al (14) observed false positive Pv-pLDH lines in 6/9 RDTs (including two- three- and four-band RDTs). They occurred in the individual RDT brands at frequencies ranging from 8.2% to 29.1%. For 19/85 samples, at least two RDT brands generated a false positive Pv-pLDH line. This is of concern as P. falciparum and P. vivax are co-circulating in many regions. The diagnosis of life-threatening P. falciparum malaria may be missed (two-band Pv-pLDH RDT), or the patient may be treated incorrectly with primaquine (three- or four-band RDTs).

Seasonal variations in relation with malaria prevalence can be explained with increased density of vector population during the monsoon and autumn, with increase in breeding fields and favorable conditions for mosquitoes. In present study, nineteen (0.18%) donors were found to be malaria antigen positive by RDT, of which only three (15.8%) were confirmed by microscopy. None of the donor had given a history of...
fever/malaria during pre-donation screening. Seasonal variations were observed. Although RDTs make an important tool for malaria testing, peripheral smear microscopy continues to be the gold standard diagnostic test for malaria diagnosis.

As per experience of many researches and a few published studies, commercially available RDTs lack the consistency, quality control and performance capabilities as claimed by the manufacturers making their use ineffective or potentially dangerous[15].

These data are also in accordance of present study, only three donors tested positive for malaria by microscopic examination of peripheral smear out of nineteen malaria antigen positive donors by RDTs, showing high prevalence of false positive results, concluding that microscopy on peripheral smear is still the most reliable method to diagnose malaria.

**Conclusion**

In present study, 19 (0.18%) donors were found to be malaria antigen positive by RDT, of which only three (15.8%) were confirmed by microscopy. None of the donor had given a history of fever/malaria during pre-donation screening. In our study only three donors tested positive for malaria by microscopic examination of peripheral smear as compared to nineteen malaria antigen positive donors by RDTs, showing high prevalence of false positive results, concluding that microscopy on peripheral smear is still the most reliable method to diagnose malaria.

Commercially available RDTs lack the consistency, quality control, high false positive results and performance capabilities as claimed by the manufacturers making their use ineffective or potentially dangerous.

**Inference-** Although RDT is an important tool for malaria testing in rural settings, we suggest the diagnosis must be confirmed with microscopy method. RDTs can be an important tool for malaria testing, peripheral smear microscopy continues to be the gold standard diagnostic test for malaria diagnosis.

**Funding:** Nil, **Conflict of interest:** None initiated

**Permission from IRB:** Yes

**Ethical approval:** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed consent:** Informed consent was obtained from all individual participants included in the study.

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How to cite this article?

Jain R, Jain P, Kashiv M, P. Desai, U. Chudgar, N. Choudhury, V.K. Mahadik. Comparative evaluation of bivalent malaria rapid diagnostic tests versus traditional microscopy method in assessment of malaria in blood donors at a tertiary care teaching hospital and regional blood transfusion centre in central India. Trop J Path Micro 2018; 4(5):421-426.doi:10.17511/jopm.2018.i5.09