

Diagnosis of Malaria among Blood Donors in Port Harcourt, Nigeria: Microscopy or Rapid Diagnostic Tests?

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Abstract

The two most common techniques available for diagnosis of malaria in Nigeria are microscopy and rapid diagnostic tests (RDTs). However, in diagnosing asymptomatic *Plasmodium* parasitaemia among donors, the preferred technique should not only be timely but must not compromise diagnosis. It should be more sensitive with minimal chances of false negative results. This study was carried out to compare microscopy and RDTs as effective tools for diagnosis of *Plasmodium* parasitaemia among blood donors in Port Harcourt. There were 200 donors involved in the study among which 135 (67.5%) tested positive and 65 (32.5%) tested negative on microscopy whereas 30 (15.0%) tested positive and 170 (85.0%) tested negative to RDT with a statistically significant difference between both techniques. Data were analysed using the statistical package *epi-info version 7.02*. We therefore recommend that microscopy using giemsa-stained blood films remain the method of choice for diagnosis of *Plasmodium* parasitaemia among blood donors to prevent the spread of transfusion malaria.

Keywords

Blood Donors, Microscopy, Rapid Diagnostic Tests

1. Introduction

Malaria is a common but highly fatal febrile illness caused by parasites of the genus *Plasmodium*. There are currently five species of the parasite that are known to cause infections in humans. *Plasmodium falciparum*

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which is the specie known to cause severe malaria that is associated with a high mortality rate is prevalent in Nigeria. It is much more severe in clinical presentation and can be fatal within a few hours of the first symptoms [1].

The World malaria report 2014 reports about 198 million cases and 584,000 deaths from malaria (mostly among African children) and 97 countries with ongoing malaria transmission [2]. In the Niger delta region of Nigeria, Erhabor and colleagues recorded a 10.2% prevalence of *Plasmodium* parasitemia among blood donors [3] while other researchers across Nigeria and Africa have reported prevalence rates ranging from 13% to 77.4% [4]-[7].

Transfusion malaria which is malaria transmitted by transfusion of blood from infected donors is one of the most common transfusion-transmissible infections [8] [9]. Most donors implicated in transfusion-transmitted malaria live in malarious areas and so are predominantly semi-immune with very low parasite loads and the estimated infectious dose is 1 to 10 parasites per unit of blood [8].

Microscopic examination of stained blood smears remains the “gold standard” for detection of malaria parasitemia. The sensitivity of this method can be excellent, with detection of malaria parasite densities as low as 5 to 10 parasites/ μ l of blood [10]. Microscopy permits determination of the infecting species, the stage of the circulating parasites and monitoring of the parasitological response to anti-malarial therapy as well as the determination of circulating parasite density which may aid in prognosis [11]. In addition, examining an individual sample is relatively inexpensive and smears for malaria parasite detection provide a permanent record for quality assessment of the microscopic diagnosis.

Despite these strengths, microscopy possesses a number of limitations viz: the procedure is labor-intensive and time-consuming and variability in stains used and techniques employed to collect and process blood affects slide interpretation [11] [12]. Mixed infections may also be missed, especially when *P. malariae* or *P. ovale* parasites are present, as their densities are often low in comparison to that of *P. falciparum*. Diagnostic errors in microscopy however, more commonly occur for low-density parasitemias (10 to 100 parasites/ μ l of blood), but errors of quantification can also occur with higher densities of $>5000/\mu$ l and especially $>20,000/\mu$ l of blood [13]. Accurate microscopic diagnosis is however, a skill that is mastered with extended training and experience [13].

Newer technological methods have emerged as alternatives to microscopy and they include antigen detection techniques, serology for antibodies, fluorescence microscopy (Quantitative buffy coat), flow cytometry, molecular amplification assays, and laser desorption mass spectrometry [11].

Malaria Rapid Diagnostic Tests (RDTs) employ lateral-flow immunochromatographic technique [14] [15].

The characteristics of the malaria antigen target and the detection antibodies are vital to understanding the performance of RDTs. Malaria antigens currently used as diagnostic targets are either specific to a *Plasmodium* specie or cut across the human *Plasmodium* species. *P. falciparum*-specific monoclonal antibodies have been developed for histidine-rich protein 2 (HRP-2) [16] [17] while Targets conserved across all human *Plasmodium* species (pan-*Plasmodium* antigens) have been identified on *Plasmodium* lactate dehydrogenase (PLDH) and aldolase enzymes [18] [19].

RDTs being simple to perform can be used by unskilled persons without formal medical laboratory training or even by travellers for self-diagnosis and treatment [11] [20]. This is a major advantage of RDTs in resource poor settings like ours.

Limitations of RDTs include the ability of these tests to give false positive and false negative results due to the persistence of the target antigens, their inability to detect mixed infections, inability to distinguish every specie of *Plasmodium*, failure to detect infections with low but clinically relevant concentrations of parasites (such as in asymptomatic infections in donors) and limited ability to monitor responses to therapy [21] [22].

This study was carried out to compare Giemsa-stained blood film microscopy and malaria rapid diagnostic test as methods for detection of *Plasmodium* parasitaemia among blood donors in Port Harcourt.

2. Materials and Methods

This study was carried out in the University of Port Harcourt Teaching Hospital located in Port Harcourt in the South-south geopolitical zone and Niger delta region of Nigeria. The University of Port Harcourt Teaching Hospital is one of the only two tertiary health institutions in Rivers state that serves a large proportion of people living in Southern Nigeria.

Ethical approval was obtained from the ethical committee of the hospital before commencement.

Two hundred blood donors were studied. From each of these, 2 ml of venous blood was collected using an

Ethylene Diamine Tetra-acetic Acid (EDTA)-containing vacutainer tube and needle. Thick and thin blood smears were made from each sample, allowed to air-dry and fixed in methanol. All smears were then stained with 10% Giemsa solution using standard procedure and examined microscopically under oil immersion. Asexual stages of *Plasmodium* parasites were identified where present and reported as positive.

Each sample was also subjected to malaria RDT using the SD BIOLINE Malaria Ag P.f Test kit (Standard Diagnostics) according to manufacturer's instructions. Microscopy and RDT were performed by different technicians and each was blinded from the other.

Data obtained was analyzed using the statistical package *Epi-info version 7.02*. The level of significance was set at 0.05.

3. Results

The mean donor age was 32.13 ± 6.25 (mean \pm SD) with a range of twenty-one (21) to forty-five (45) years.

The majority of donors accounting for 97.5% (195) were male while females accounted for just 2.5% (5) of all donors screened with the difference being significant statistically ($\chi^2 = 361.00$, p -value = 0.001) (Table 1).

Fifty-five percent (55%) of donors were aged between twenty-six and thirty-five years, twenty-five percent (25%) between thirty-six and forty-five years while only twenty percent (20%) were ≤ 25 years of age (Table 1).

The prevalence of *Plasmodium* parasitaemia using Giemsa-stained blood films and that using RDTs were compared and differed widely. One hundred and thirty-five donors (67.5%) tested positive and sixty-five (32.5%) tested negative to microscopy (Table 2) whereas thirty (15.0%) tested positive and one hundred and seventy (85.0%) tested negative to RDT (Table 3). This shows that over 50% of infected persons in this study were missed by the RDT and that is a very large proportion.

Table 4 shows the comparative analysis between both diagnostic techniques and reveals that the difference was statistically significant ($\chi^2 = 111.58$, $p = 0.001$).

4. Discussion

The emergency donation of blood still occurs quite commonly in a large proportion of Nigerian health institu-

Table 1. Age and sex distribution of donors.

Characteristics	Frequency (%)	Chi-square (χ^2)	p -value
a) Sex Distribution of Donors			
Male	195 (97.5)	131.64	0.001
Female	5 (2.5)		
Total	200 (100.0)		
b) Age Distribution of Donors			
≤ 25	40 (20.0)	64.50	0.001
26 - 35	110 (55.0)		
36 - 45	50 (25.0)		
Total	200 (100.0)		

Table 2. Prevalence of plasmodium parasitaemia among blood donors using microscopy.

MP (Using Giemsa stain)	Frequency	Percent
Positive	135	67.50%
Negative	65	32.50%
Total	200	100.00%

MP = Malaria parasite.

Table 3. Prevalence of plasmodium parasitaemia among blood donors using RDT.

MP (using RDT)	Frequency	Percent
Positive	30	15.00%
Negative	170	85.00%
Total	200	100.00%

MP = Malaria parasite.

Table 4. Comparison between Malaria RDT and Microscopy.

Diagnosis of <i>Plasmodium</i> Parasitaemia among Blood Donors	RDT N = 200 (%)	Giemsa Stain N = 200 (%)	CHI-Square (χ^2)	p-Value
Positive	30 (15.00)	135 (67.50)	111.58	0.001*
Negative	170 (85.00)	65 (32.50)		

*Significant ($p < 0.05$).

tions because the culture of routine blood donation is still quite poor among Nigerians. The method employed for pre-donation testing for malaria parasite in these situations is indeed critical because time would be of the essence; however, the balance between reduced testing time (reduced turnaround time) and accuracy must be maintained without impairing the quality of the diagnostic process.

Although Rapid diagnostic tests would seem the most feasible technique for diagnosis of malaria under such emergency settings due to the short testing time/reduced turnaround time associated with them, our findings reveal that microscopy using the giemsa-stained blood film leads to a much higher detection rate and therefore far more useful for diagnosis of asymptomatic *Plasmodium* parasitaemia among blood donors as this will greatly reduce the chances of missed diagnoses which may then result in transfusion malaria.

This finding is supportive of the fact that microscopy remains the gold standard for diagnosis of malaria as it is indeed more sensitive than RDTs. Some researchers have however, considered this a function of over-diagnosis of malaria using the giemsa-stained blood films due to human errors [23]. It is important to note that the rate of false positive results is greatly reduced when the microscopist is adequately trained and experienced [24]. This difference is probably due to geographical differences which account for the low prevalence of *Plasmodium* parasitaemia in that area.

The Centers for Disease Control and prevention (CDC) of the United States of America recommends that the need for malaria microscopy should not be eliminated by the use of RDT as the latter may not be able to detect some infections with lower numbers of malaria parasites circulating in the patient's bloodstream such as is most likely in asymptomatic parasitaemia seen in some donors and also because there is insufficient data available to determine the ability of the RDT to detect the 2 less common species of malaria, *P. ovale* and *P. malariae* [24].

Therefore all negative RDTs must be followed by microscopy to confirm the result. In addition, all positive RDTs also should be followed by microscopy because microscopy is needed to determine the species of Plasmodium that was detected by the RDT and for prognostication [24].

In addition, the cost effectiveness of the use of RDTs in Nigeria must be considered. The World Health Organization has stated that the cost-effectiveness of RDTs in areas of high prevalence such as Sub-Saharan Africa, in particular, has been controversial they are adjudged to be cost-effective in areas with prevalence of less than 20% which doesn't apply to Nigeria [25].

5. Conclusion and Recommendations

Though convenient and easy to use, RDTs still possess limitations that make their use for prevention of transfusion malaria limited in efficacy therefore since we cannot afford to continue to fuel transmission of malaria via blood transfusions, we recommend that microscopic examination of giemsa-stained blood films remain the method of choice for diagnosis of sub-clinical *Plasmodium* parasitaemia among blood donors. Encouraging the practice of Routine blood donations would afford the blood bank the time required for malaria microscopic diagnosis.

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