ORIGINAL ARTICLE

Diagnostic Accuracy of Elisa for Malarial Antibody and rapid diagnostic test for detection of Malarial Parasite in blood donors

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ABSTRACT

Aim: To find the diagnostic accuracy of Malarial antibody ELISA and RDT (rapid diagnostic test) for detection of malarial parasite in healthy blood donors, by taking blood film microscopy as reference standard
Study design: Retrospective Cross-sectional analytical study
Methodology: One thousand, three hundred and twenty-seven (1327) samples from eligible blood donors presenting in blood bank of Shaikh Zayed Hospital Lahore, Pakistan, fulfilling the inclusion criteria were evaluated in the study. We compared diagnostic accuracy (sensitivity, specificity, positive predictive value & negative predictive value) of Antibody ELISA and RDT with Blood Fil Microscopy as reference standard.
Results: Total 1327 no. donors were tested. The sensitivity and specificity of Antibody ELISA is 46.15% & 82.88% respectively, while the sensitivity and specificity of RDT is 84.62% and 99.92% respectively.
Conclusion: As malaria is disease to be tested in blood donors according to WHO guidelines, it is very important to establish a proper technique for it and in our study, it is concluded that RDT is more accurate than Antibody ELISA for the detection of malaria in potential blood donors.
Keywords: Malaria, Antibody ELISA, RDT(Rapid Diagnostic test), Blood Donors, Blood Film Microscopy

INTRODUCTION

Malaria is a parasitic disease and it basically invades red blood cells. There are 106 countries where this disease is endemic and it has led to 655,000 deaths in 2010, in which 86% were children[1]. Malaria transmission is by the bite of an infected mosquito i.e., female *Anopheles*[2].when the patient is infected with this disease he develops fever and some other non-specific symptoms, but in the case of P. falciparum infection the patients is prone to complications and can quickly progress to vital organ failure or dysfunction pulmonary edema, coma or shock. Some cases of plasmodium Vivax infection with severe malaria have also been seen. On the other side there is asymptomatic malaria infection in some cases[1].

Malaria is acute but sometimes chronic febrile health issue. Female Anopheles Mosquito inoculates it's sporozoites in the blood stream of the human. There are four species of malaria causing mosquito i.e., *Plasmodium falciparum, Plasmodium malariae. Plasmodium vivax, Plasmodium ovale*. These four species are responsible for causing malaria in humans' beings. Malarial infection is found in human all over the world. According to the assessment of World Health Organization, globally 40% population is at great risk of malarial infection. Almost 300–500 million populations are infected with the malarial infection. Pakistan is highly prevalent in malarial infection. According to the directorate of Malaria Control one person per thousand is infected with the malaria infection[3].

For cases when malaria is transmitted via transfusion of blood to a potential recipient who is non-immune previously, this transfusion of infected blood can rapidly progress and may lead to significant morbidity and mortality, especially in cases when diagnosis is delayed[4]. Light microscopy on thick and thin film& Rapid diagnostic tests (RDT) by immunochromatography technique are still approved for diagnosis of malaria and guide the clinical management.

The majority of cases in Afghanistan and Pakistan, and almost all cases in Iran and Iraq are due to Plasmodium Vivax infection. The assessed number of yearly malaria cases in Pakistan in 1.5 million. In 2006, the Malaria Disease Surveillance Programme in Pakistan enlisted 3.5 million slides arranged for malarial parasite and 127,825 affirmed cases of malaria with annual parasite rate (API) of 0.8 per 1000 populace[6].

In malarial endemic zones, tainted blood donors act as a origin of infection to blood recipients, which can antagonistically influence their prognosis. Between September 2015 and June 2016, a prospective study was conducted, total 1240 potential blood givers were selected. The pre-dominance of malarial parasite in this study was found to be 8.1%. The rapid diagnostic test had a sensitivity of 88%, specificity of 99.1% and negative predictive value of 99.0%. The antibody ELISA has a sensitivity of 69.9% and specificity of 80.3%[7].

Transfusion-transmitted malaria was first reported in 1911. Third transmission path of Plasmodium is also important to acknowledge. all five human malaria parasites that is *Plasmodium Falciparum, Plasmodium Malariae, Plasmodium Ovale, Plasmodium vivax and Plasmodium Knowlesi*, may also be transmitted via transfusion of blood. Transmission of malaria via transfusion of blood or blood products is a lethal complication and it causes a continuous

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risk for blood banks. The recipient's malaria diagnosis is not expected, and is thus often easily missed. Malarial parasites can survive for days or weeks at refrigerator temperature (2-4°C) in stored red blood cells, so it is of utmost importance to exclude all blood donors at risk[8]. In later advances antibody ELISA is additionally performed, based on binding of anti-Plasmodium antibodies present in serum or plasma tests to the antigens immobilized on plates .Schedule screening of all blood donations would avoid tainted blood transfusion and increase the donation's security for vulnerable and immunocompromised recipients, such as children and pregnant ladies and old age patients[8].

The objective of the study was to find the diagnostic accuracy of Malarial antibody ELISA and RDT (rapid diagnostic test) for detection of malarial parasite in potential blood donors, by taking blood film microscopy as gold standard.

METHODOLOGY

This retrospective Cross-sectional analytical study Blood Bank, Shaikh Zayed Hospital Lahore from 15th May 2019 to 14thNovember 2019. Sampling technique used was Non-probability Consecutive

All blood donors of both genders at the age of >18yrs to <65yrs fulfilling donor selection criteria as per WHO guidelines were included in the study. Patient having <50 kg with history of intake of antimalarial drugs in last 6 months were excluded from the study.

TEST METHODS: Two index tests were evaluated for the detection of target condition which is malaria

Rapid Diagnostic Test (RDT): There is antigen antibody reaction on nitrocellulose strip using Histidine rich protein 2 (HRP-2). Two or three distinct colored lines appear in which one is control line and rest of two are in pan line region and plasmodium falciparum region.

Antibody ELISA: A qualitative immune-enzymatic determination of antibodies against plasmodium is based on ELISA technique in the serum. The ELISA malaria antibody test is based on binding of anti-Plasmodium antibodies present in a serum sample to antigens immobilized on 96-well plates The antibody titer of more than cut off value of 10.0 is considered positive.

Microscopy: Different species and stages of malarial parasite are seen under the microscope in Giemsa stained thick and thin smears. Result is considered positive on presence of any stage of any species of Malarial parasite on either thick or thin smears.

Sensitivity: $\frac{\text{True positive}}{\text{True Positive + False Negative}}$

Specificity: $\frac{\text{True Negative}}{\text{True Negative + False Positive}}$

Positive Predictive Value (PPV): $\frac{\text{True Positive}}{\text{True Positive + False Positive}}$

Negative Predictive Value (NPV): $\frac{\text{True Negative}}{\text{True Negative + False Negative}}$

Total 1362 blood donors presented in blood bank, out which 35 donors were deferred on basis of history and not fulfilling the inclusion criteria. One Thousand, three hundred and twenty-seven (1327) samples were collected in EDTA vacutainer from eligible blood donors presenting in blood bank of Shaikh Zayed Hospital Lahore, Pakistan, from 15th May 2019 to 14th November 2019**.** After informed consent and fulfilling the donor assessment criteria as per WHO guidelines, socio-demographic data of selected donors like name, age, gender was recorded and screening tests both index tests and reference standard for Malarial parasite detection were performed on these donors. RDT was performed on Fastep MAL-W23M (malaria P falciparum/pan rapid test device) taking 5-10µl of blood. Blood film microscopy was done on Olympus microscope by making both thin and thick smears of blood either of them using 5-10µl of blood. Antibody ELISA was done on Diasorin ETI Max 3000 by using 2-5µl of blood).

Data was collected and analyzed on SPSS v.22. Data analysis included mean and standard deviation of age. The Frequency of disease in our population. Specificity, sensitivity, positive and negative predictive value and diagnostic accuracy of Antibody ELISA and RDT were also calculated with taking microscopy as reference standard. Data was also stratified according to age in to two groups. Post stratification a 2x2 table was applied to calculate sensitivity, specificity, PPV, NPV and Diagnostic Accuracy.

RESULTS

A total of 1327sample from blood donors were included in this study. Donors who fulfilled the criteria were selected after initial assessment according to donor assessment form. Each individual was tested for the presence of malarial parasite. A total of three tests were performed on every individual, where each test was executed individually on each sample of donor and results were carefully recorded and data was entered into SPSS Version 22 for its analysis. The performed tests are listed below:

- Antibody ELISA
- RDT (Rapid Diagnostic Test)
- Blood film microscopy by thin and thin film

On the basis of these 3 tests, the data was summarized and following parameters were recorded;

- Mean and standard deviation of age
- Prevalence of disease
- Sensitivity and Specificity
- Positive and negative predictive value

Total 1327 samples were analyzed the mean age of the donors was 26.4±6.4yrs. The Disease prevalence was 0.98% in the healthy donors presenting in blood bank of Shaikh Zayed Hospital Lahore, Pakistan

Antibody ELISA when performed on 1327 blood samples showed total 231(17.4%) positive cases and 1096(82.6%) negative cases. The total percentage of positive cases on Rapid Diagnostic tests was 0.9% (12 cases) and percentage of negative cases was 1515(99.1%)

When Reference standard i.e., blood film microscopy on thick and thin smear was performed there were total 13(0.98%) positive cases and 1314(99%) negative cases.

Comparison of antibody ELISA and microscopy showed that there were 06 donors who were true positive, 07 donors were false negative, 1089 donors were true negative and 225 donors were false positive. This shows that few active cases were also missed by this technique. This can occur if the test is performed early in the disease

course, when no antibody has been formed against malarial antigen (Table1,2).

When the results of RDT were compared with reference standard i-e microscopy there were 11 donors who were true positive, 02 donors were false negative, 1313 donors were true negative and 01 donor was false positive. The results of RDT are quite comparable to our reference standard and out of total 13 true positive cases 11 were detected by RDT. On basis of this data collected and analyzed the sensitivity, specificity, positive predictive value and negative predictive value were calculated by 2x2 table (Table 3).

Table 1: Comparison of results of Antibody ELISA with Microscopy (n= 1327) (p<0.001)

		Microscopy		Total
		Positive	Negative	
Antibody ELISA	Positive	06	225	231
	Negative	07	1089	1096
Total		13	1314	1327

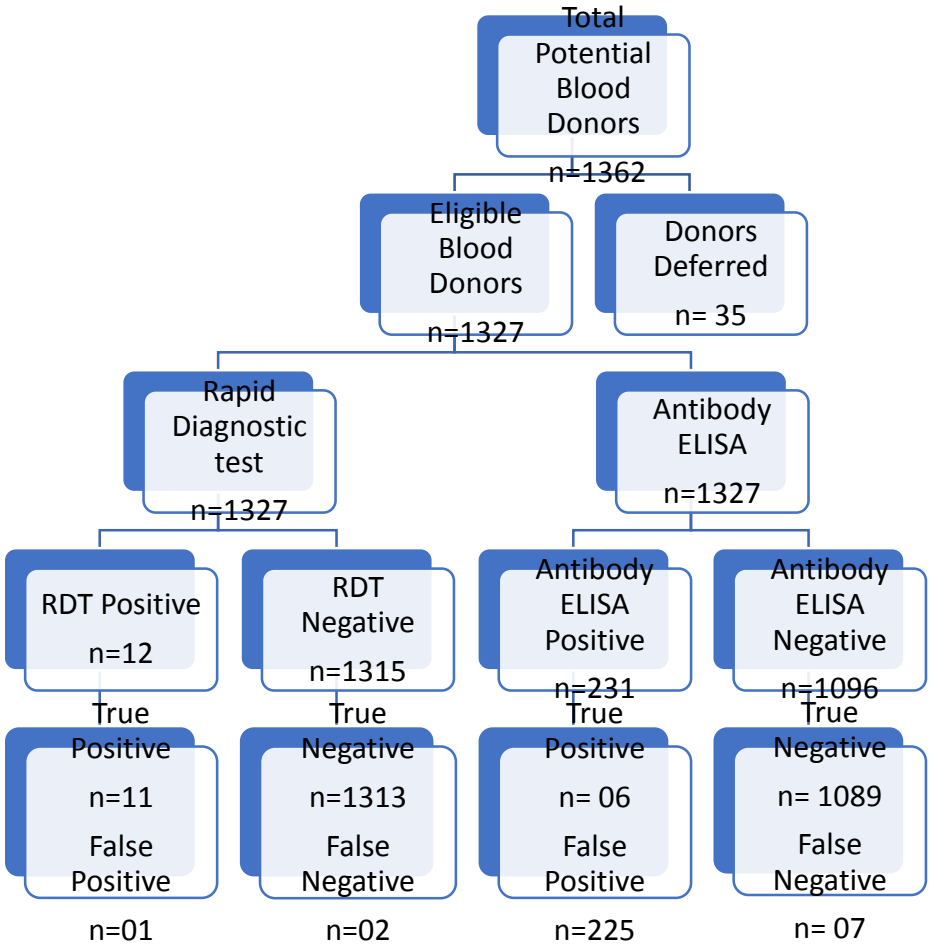
Table 2: Comparison of RDT results with Microscopy (n=1327) (p<0.001)

		Microscopy		Total
		Positive	Negative	
RDT	Positive	**11**	**01**	**12**
	Negative	**02**	**1313**	**1315**
Total		**13**	**1314**	**1327**

Table 3: Sensitivity, Specificity, PPV and NPV for RDT and Antibody ELISA (95% CI)

	RDT	Antibody ELISA
Sensitivity	84.62 %	46.15 %
Specificity	99.92 %	82.88 %
Positive predictive Value	91.67 %	2.6 %
Negative Predictive Value	99.85 %	99.36%

Fig:1: Total number of donor selected and tested



DISCUSSION

Transfusion transmitted diseases (TTI) are a vital issue related with transfusion of blood. precise gauges of the hazard of TTIs are basic for observing the provision of safe blood and blood products and assessing the adequacy of the presently utilized screening methods. Transfusion transmitted diseases are an extraordinary concern of security for patients. The immensity of the TTI shifts from nation to nation depends upon TTIs loads in that specific populace from where blood units are sourced. The dominant parts of the issues are due to the pre-dominance of asymptomatic carriers within the society, as well as blood donations amid the window period of diseases. Hiding the medical history by prisoners, paid or professional blood donors, who broadly exist in developing

countries, also postures a huge risk to safe blood provision. There's a long list of infections, parasite and microbes which can be transmitted through blood transfusion. Among them, critical transfusion transmitted infections are human immunodeficiency infection (HIV-I/II), hepatitis B infection (HBV), hepatitis C infection (HCV), syphilis contamination by spirochaetes and transfusion related malarial parasite[9].

Within the vast larger parts of tropical and sub-tropical districts of the world, malaria remains the foremost complex and over powering wellbeing issue, confronting humankind with 300 to 500 million cases and 2 to 3 million deaths per year. Actuated malaria by blood transfusion was to begin with detailed in 1911 and it is well built up that all four human malarial parasite species i-e *plasmodium falciparum, plasmodium ovale, plasmodium vivax and plasmodium malariae* may be transfusion transmitted[10].

It was observed during our study that sensitivity and specificity of antibody ELISA for malarial parasite is 46.15% and 82.88 respectively. whereas sensitivity and specificity of Rapid Diagnostic Test (RDT) is 84.62% and 99.92% respectively. With these results it is easily concluded that RDT is considered more accurate than antibody ELISA. It is also important here, that Pakistan is an endemic area for malarial parasite and increase number of false positive results shown by Antibody ELISA will lead to rejections and deferrals ofa large number of potential donors and will make this technique less reliable for endemic countries.

The results of our study are somewhat comparable to another study conducted in Beau, Cameroon in which the sensitivity and specificity of Antibody ELISA is 69.9% & 80.3% respectively and sensitivity and specificity of RDT is 88% and 99.1% respectively[7].

The results of another study done in Senegal is also somewhat comparable to our study with sensitivity & specificity of Antibody ELISA 88% and 87% respectively and sensitivity & specificity of RDT is 89% & 100% respectively[11].

In the endemic areas for malaria, epidemiological studies have detailed a pre-dominance of malaria among potential blood donors to run between 1% and >50 %. the chance of malaria disease transference through transfusion of blood is accounted for by the perseverance of malarial parasite within the blood.

Every species can survive in the blood for quite a long time and differs from species to species that is in case of Plasmodium ovale it can survive for nearly three years in blood. For plasmodium vivax the time of survival in blood is almost one year and the Plasmodium malariae remains in the blood for quite longer periods of time before causing any symptoms for the clinical diagnosis of malaria. The identification of parasite in geimsa stained thick and thin films by light microscopy is considered to be the gold standard for malaria diagnosis and it is as of now the foremost broadly utilized procedure for the conclusion of malaria in endemic regions[7].

Form our study it is evident that as Pakistan is an endemic country for Malaria and the sensitivity of Antibody ELISA is too low to detect the true positive infected donors. The ratio of false positive results for Antibody ELISA is quite high and this will lead to deferral / rejection of a large number of potential donors. However, the results of RDT are very much comparable to our gold standard i-e blood film microscopy.

Rapid Diagnostic test is quite easy to perform and can be done on large number of samples in a small period of time, more over RDT can also be performed in remote areas without any specialized equipment and staff. The results of RDT are also much comparable and correlate with our gold standard i-e- blood film microscopy.

CONCLUSION

It is inferred in the end that as malaria is one of an important transfusion transmitted infections and according to WHO it should be tested and every donor must be screened along with other infectious diseases, so it is important to establish a reliable and accurate and accessible technique to decreases the chances of malarial transmission by blood donations.

In our study two of the techniques i-e antibody ELISA and RDT are being tested against gold standard which is blood film microscopy. It is inferred from our study that RDT is a much better, accurate and sensitive technique than antibody ELISA in our region, because Pakistan is an endemic country for malarial parasite and Antibody ELISA will give a large number of false positive results which will lead to deferral of a large number of potential donors.

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