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Malaria Screening Among Voluntary Blood Donors to Find Out The Prevalence -to Evaluate the Sensitivity of Different Technique

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Abstract*: Malaria can be transmitted by transfusion of blood from infected donors and was first reported in 1911. This study was done to know the prevalence of malaria among voluntary blood donors, to compare the various screening methods and to find out the most sensitive technique to screen Malarial parasite among voluntary blood donors. A cross sectional study was conducted to study the prevalence of malaria among voluntary blood donors. Two ml of EDTA blood sample was collected and used for screening malaria parasite by Peripheral blood smear, Quantitative Buffy Coat method, Rapid Card Test. The prevalence of malaria among the voluntary blood donors was 0.4%. Among 250 donors, one of the donor blood samples was found to be positive by Microscopy, QBC and RDT. The species identified was P.vivax. The sensitivity and specificity of QBC and RDT were 100% with respect to the gold standard microscopy. Hence, to prevent transfusion transmitted malaria in endemic areas like India screening of malaria by Rapid Detection Test may be included along with microscopy.*

Keywords*: Transfusion-Transmitted Infections(TTI), Peripheral blood smear, Quantitative Buffy Coat method, Rapid Card Test.*

I. Introduction

Blood Transfusion Service is a vital part of the National Health Service and there is no substitute for human blood and its components. 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 per million cases for non-endemic countries to 50 or more cases per million in endemic areas[3]. The microscopic detection of blood though considered the gold standard for malaria diagnosis for decades, it is quite labor intensive and require adequate technical skill and man power. This had spurred the development of other microscopic malarial and rapid detection test based on the detection of malarial parasite antigen in the whole blood4. There are many studies available regarding transfusion transmitted malaria and this study was undertaken to study the prevalence of malaria among voluntary blood donors, to compare the various screening methods and to find out the most sensitive technique to screen malarial parasite among voluntary blood donors.

II. Material and Methods

A cross sectional study was conducted in Voluntary Blood Donors (VBD) from the department of Transfusion medicine of the Tamil Nadu Dr.M.G.R.Medical University, Chennai, from June 2011 to August 2012 and human ethical clearance was obtained from the IRB of the Tamil Nadu Dr.M.G.R.Medical University. Randomly selected 250 eligible blood donors with no history of fever in the past 3 months were included in this study. After obtaining the informed consent from the donor, a semi-structured questionnaire (standard Voluntary donor form) was used to obtain information on demographic details and other risk factors such as previous history of malaria, treatment taken for malaria etc. 2ml of EDTA blood sample was collected and used for screening malaria parasite by Peripheral blood smear, Quantitative Buffy Coat method and Rapid Card Test.

2.1. Peripheral Smear Method

QMicroscopic examination of the blood films is known as the current universal "gold standard". Preparation of thick and thin Blood Film was done by standard protocol. The stained film is examined under oil immersion microscope. If the parasites are seen in the thick film, the thin film should be examined to determine the species. Parasitemia is estimated by finding the number of parasitized RBC in 10,000 RBC.

2.2. Quantitative Buffy Coat Method

Quantitative Buffy coat method was done with acridine orange dye which stains nucleus and cytoplasm by binding with DNA and RNA. The presence of parasite and its morphology examined under fluorescent microscopy after excited at 480 nm, the nucleus show yellowish green and the cytoplasm show bright red fluorescence. The presence of malarial parasite DNA and RNA is identified by the green and orange fluorescence emitted respectively. Every tube was examined until the presence of parasite or for maximum five minutes.

2.3. Rapid Card Test

The test kit ready to use after bringing it to the room temperature, port A in the test kit filled with 5 microlitre of anticoagulated blood by micropipette and allowing it mix evenly in port B two drops of buffer solution delivered vertically with the help of plastic dropper. The results are interpreted after 20 minutes. Two pink-purple bands in the Pf and Pan region in the test window T along with control band indicate positive for plasmodium falciparum or mixed infection. Only one pink-purple band in control indicates negative result and one pink-purple band in Pan region at test window T along with control indicate positive for other species.

III. Results

This cross sectional study was conducted on 250 voluntary blood donors. In the present study, the most common age group of voluntary blood donors were less than 22 years (30.4%) with overall sex distribution of 94% males and 6% females. The prevalence of malaria among the voluntary blood donors was 0.4%. Among 250 donors, one of the donor blood samples was found to be positive by Microscopy, QBC and RDT. The species identified was P.vivax. Positive malaria was observed in male in the age group of 22yrs. The sensitivity and specificity of QBC and RDT were 100% with respect to the gold standard microscopy.

Comparative Of Microscopy, QBC And Card Test For Malaria Screening Among Voluntary Blood Donors

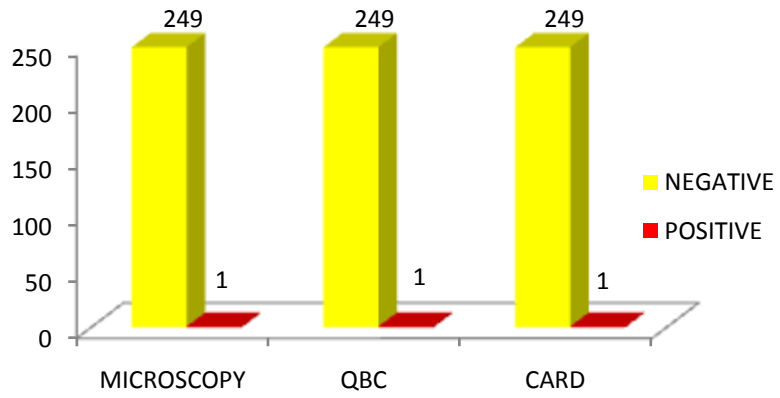

Fig. 1

In our study of the 250 donors, there was 1 donor postive for malaria which was identified by Microscopy,Rapid Card and QBC methods.The species of malarial parasite identified by the above methods was plasmodium vivax.

The Table below shows the malaria positivity with the previous history of malarial infection (four months back). This shows that there was a statistically significant association by fisher's exact test (p-value-0.004)

Correlation between Donor with Previous History of Malaria and Serological Positivity

Past history of malaria			Malaria Seropositivity			Chi-Square	p- Value (FISHER'S Exact Test)
Present	Absent	Total	Positive	Negative	Total		
1	249	250	1	249	250	0.000	0.004

Table 1

Among the various tests for detection of malarial parasites, Microscopy has been considered as the Gold Standard method. In our study, QBC and RDT were compared with the gold standard microscopy.

Comparison of diagnostic test with gold standard

QBC	MICROSCOPY		TOTAL
	POSITIVE	NEGATIVE	
POSITIVE	1	0	1
NEGATIVE	0	249	249
TOTAL	1	249	250

Table 2

Since we found only one positive among the entire test the Sensitivity and Specificity of the QBC were 100% with respect to the gold standard test microscopy.

Sensitivity and specificity of rapid diagnostic test with respect to Microscopy

RDT	MICROSCOPY		TOTAL
	POSITIVE	NEGATIVE	
POSITIVE	1	0	1
NEGATIVE	0	249	249
TOTAL	1	249	250

Table 3

The Sensitivity and Specificity of the Rapid Diagnostic Test were 100% with respect to the Microscopy method. In the present study, sensitivity and specificity of QBC and RDT were 100% with respect to the gold standard
microscopy.

IV. Discussion

Voluntary blood donors with asymptomatic infection of malarial parasite contribute to the risk of Transfusion Transmitted Malaria. Out of 250 donors in this study one was found to be positive for HBsAg and another donor was found to be positive for malaria. Gupta et al reported increased seropositivity of HIV, Anti HCV and HBsAg in replacement donors when compared to voluntary donors.[5] In the present study, among the 250 voluntary blood donors, one donor was positive for malaria by Microscopy, QBC and Antigen Detection Rapid Diagnostic Test. The prevalence rate of malaria in our donor study population was 0.4%. Similar to our study, Bahadur et al 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.[4] In concordance with our study, Choudry N et al. in their study conducted in northern India found the prevalence rate among voluntary blood donors to be 0.35% by antigen detection method.[6] Lim CS et al in their study conducted in Korea among blood donors found the malarial antigen prevalence rate to be 1.7% by PCR method. In comparison to our study, the high antigen prevalence rate in their study was due to highly sensitive detection method.[7] Anju Dubey et al 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, as more sensitive malaria screening methods like RDT, malaria antigen testing by ELISA are available.[8] This is similar to our study with respect to the past history of malaria and positive antigen detection RDT. However, in our study microscopy also found to be positive. In the present study, sensitivity and specificity of QBC and RDT were 100% with respect to the gold standard microscopy. SC Parija et al in their study compared microscopy (gold standard), QBC and antigen detection test and reported the sensitivity and specificity of QBC were 78.94% and 98% respectively while sensitivity and specificity of RDT were 75% and 100% respectively.[9] Mishra et al in their study reported the sensitivity and specificity of RDT for detection of P.vivax was 100% and the sensitivity and specificity of RDT for detection of P.falciparum was 96% and 100% respectively when compared with microscopy as gold standard.[10] In 2008, Bharti et al in their 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%.[11]

V. Conclusion

Among 250 voluntary blood donors in a malarial endemic area one sample was found positive by microscopy, Quantitative Buffy Coat Method and Rapid Diagnostic Test. Since intermittent asymptomatic period, recrudescence and relapse are known with P.vivax infection in an endemic area, especially in semi-immune individuals, it is imperative to screen all donors by less-laborious, less time-consuming and more sensitive methods, irrespective of a strict donor questionnaire. However, a study on large number of voluntary blood donors is necessary to arrive at a definitive conclusion. Since technically even PCR method can miss very low parasitemic load causing transfusion transmitted malaria, strict donor deferral criteria and antibody screening may be considered similar to non-endemic areas. But, this would definitely lead to shortage of donor pool and more wastage of collected units. If necessary, antibody screening positive units can be subjected to pathogen inactivation or alternatively post-transfusion chemoprophylaxis may be tried.

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