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Molecular Screening for Malaria Parasitemia among Blood Donors: A Regional Transfusion Center Study in Pakistan

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Abstract

Objective: This study was carried out to determine asymptomatic malaria parasitemia among blood donors using molecular method and frame guidelines for malaria screening in blood banks of Northern Pakistan.

Methodology: In this cross-sectional study, a total of 1000 asymptomatic blood donors were enrolled between April and September, 2019. DNA extraction, amplification and detection of targeted gene (18S small subunit rRNA) was carried out using DNA extraction kit and Real Time PCR system respectively. Any sample found positive was retested for confirmation. Malaria parasitemia was expressed as percentage using descriptive statistics.

Results: Among 1000 asymptomatic donors, all donors were males with a median age of 30.0 years. Malarial parasite was detected in 0.1% blood donors (n=1/1000). Upon retesting of positive sample by another RT-PCR system, consistent results were obtained. Donor questionnaire did not reveal any history of previous exposure.

Conclusion: Malaria appears to be very uncommon in asymptomatic male blood donors and screening by NAT would not be economical. Adding effective donor screening criteria is suggested.

Keywords: Blood safety, Malaria, Molecular testing.

Introduction

Malaria inflicts global population and an estimated 228 million new cases are reported worldwide.[1] Approximately, 60% of Pakistan population contributes to malaria burden with annual parasitic incidence (API) rate of 1.8.[2] Apicomplexan parasites of the genus *Plasmodium* are the etiological agent which disseminates by bite of infected female Anopheles mosquito during a blood meal. Most common species involved are the *Plasmodium vivax* and *Plasmodium falciparum*. Besides, transfusion of blood components contaminated with malarial parasites is also a contributing source.[3,4]

Transfusion transmitted malaria (TTM), first described by Woosley in 1911[5], has gained interest in blood banking because of conversion of hemoglobin into dysfunctional methemoglobin leading to decreased systemic delivery of oxygen accompanied by fatal complications in debilitated recipients.[6] Contaminated blood products, especially whole blood and red cell concentrates, directly release the merozoites, in the bloodstream leading to adverse outcomes as compared to vector-borne transmission where pre-erythrocytic phase confers advantage in triggering the development of protective immunity.[7]

Survival of parasites for at least 18 days at storage temperature of blood products is a well-recognized risk of TTM.[8] Microscopy is a gold standard method however, sub-patent infections characterized by low parasite density (<10 parasites/μL) escapes detection which remains a diagnostic challenge in blood banks.[9,10] Thus, appropriate screening tools are the mainstay of prevention of TTM. High-sensitive molecular methods need to be employed to detect low infectious inoculum in asymptomatic blood donors which act as reservoirs sustaining malaria transmission.[11,12] As far as we know, there is lack of data depicting the actual burden of malaria prevalence among blood donors in Pakistan. Therefore, this study was conducted using highly sensitive method such as polymerase chain reaction (PCR) to identify the risk of and burden of TTM.

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Methodology

Transfusion center based cross-sectional study was conducted from April to September 2019. Ethics Committee of the Armed Forces Institute of Transfusion (AFIT) approved the research protocol. Prospective random donors with age ranges from 18-65 years and body weight ≥ 50kg were recruited using structured questionnaire while those who were seropositive for any transfusion transmissible infections (TTIs) including, HIV, HBV, HCV and syphilis, having history of anemia, bleeding disorders, body weight <50kg and refused to participate were excluded. Written informed consent was obtained from all enrolled donors. A total of 1000 asymptomatic blood donors were included by using random sampling technique. A 5ml of whole blood sample was collected into ethylene diamine tetra acetate (EDTA) tubes from each donor following standard protocol for venipuncture. DNA was extracted using commercial DNA-extraction kit (Qiagen, Germany) according to manufacturer's instructions for real time-PCR diagnosis of malaria. DNA amplification and detection were carried out using Applied Biosystems ABI 7500 Real-Time PCR System (Thermo Fisher Scientific, USA). Primers and probe based on detecting conserved sequences of 18S small-subunit ribosomal ribonucleic acid (rRNA) genes of all *Plasmodium* species are shown in Table I. Negative and positive controls were run with each batch. Any sample found positive was retested in duplicate by Rotor gene plex RT-PCR system for confirmation and re-examination of the questionnaire filled by malaria infected donors was also performed.

Table I: Primers and Probes for detection of Malarial Parasites

Primers	Sequence
Forward	5′-ACATGGCTATGACGGGTAACG-3′
Reverse	5′-TGCCTTCCTTAGATGTGGTAGCTA-3′
Probe TaqMan®	5′-TCAGGCTCCCTCTCCGGAATCGA-3′
Reporter Dye	FAM (carboxyfluorescein) on 5′ end
Quencher dye	BHQ1 (black whole quencher) on 3′ end

Descriptive analysis was carried out using Statistical Package for Social Sciences (SPSS) version 23.0. Parasitemia was expressed as percentage (positive samples/Total samples ×100).

Results

A total of 1000 blood donors were screened for malarial parasites using RT-PCR method. Demographic characteristics showed that all donors were males and their median age was 30.0 years (ranging from 18-65 years). Highest proportion was constituted by age group 18-28 years (Table II). Among these, only one asymptomatic donor (0.1%, n=1/1000) was found to be positive for malarial parasite (Figure 1). This case was also tested in duplicate for confirmation using another RT-PCR system. Results obtained by two RT-PCR methods were consistent. Upon re-examination of the questionnaire of the malaria positive donor, no history of previous possible exposure was identified.

Table II: Demographic characteristics of enrolled donors

Variables	N(%)
Gender	
Male	1000(100%)
Female	00(0%)
Age Groups	
18-28 years	550 (55%)
29-39 years	350 (35%)
≥40 years	100 (10%)

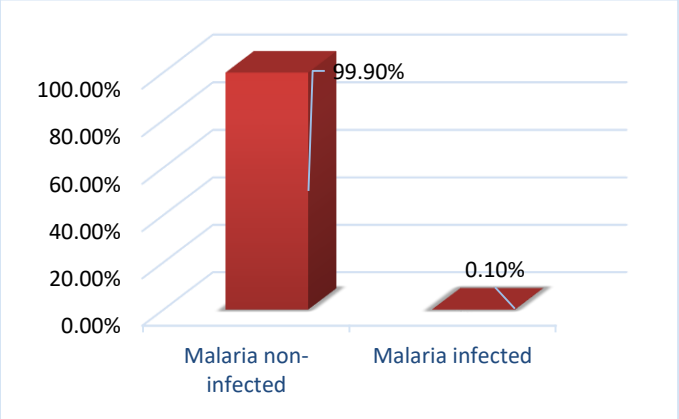

Figure 1. Distribution Frequency of Malaria Infected and Non-infected Blood donors.

Discussion

Blood transfusion remains a major risk factor in transmission of various infections. Blood transfusion services employ various serological and molecular methods to detect the presence of TTIs in order to provide safe and effective blood.[13] Pakistan is epidemiologically a moderate-malaria endemic country

Table III: Comparison of current study with previously conducted local studies

Year of Study	Place of Study	Method	TTM Prevalence	Reference No.
2019-2020	**Rawalpindi**	**RT-PCR**	**0.1%**	**present**
2018-2019	Faisalabad	CLIA	0.89%	16
2016-2018	Multan	ICT	0.0%	15
2015-2016	Lahore	ICT	0.39 %	17

ICT; Immunochromatographic technique, CLIA; Chemiluminescence microparticle immunoassay, RT-PCR; Real time polymerase chain reaction

with varying degree of endemicity in its different provinces with least epidemiology of 1.1% reported in Northern Pakistan in 2017.[3] Malaria screening is mostly performed by light microscopy and rapid detection test (RDT) which are unable to detect low parasitemia thus accurate figures of TTM prevalence needs to be investigated using highly sensitive methods in transfusion medicine. Accurate diagnosis and management of malaria is essential to avoid post-transfusion hemolysis.[14] In the present study, 0.1% asymptomatic donor population was found positive using RT-PCR method. Prevalence varies across different regions of Punjab Province, Pakistan as shown in Table III.[15-17]

To date, this was the first study which employed PCR-based testing of *Plasmodium* pathogens among blood donors. High sensitivity of PCR should be taken into consideration because sub-patent blood donors serve as a reservoir of transmission. Light microscopy of stained blood smears is widely used method in clinical diagnostic laboratories but time-consuming (15-20 minutes per microscopic observation) and laborious procedure restricts its utilization to screen large population in blood banks. Moreover, its detection limit is 50 parasites/ul whereas most asymptomatic donors have lesser parasitemia.[18] In contrast to international studies, a retrospective study conducted in India showed 0.024% prevalence rate for malarial antigen by RDT method.[19]

African countries like Ghana and Nigeria reported approximately 8% prevalence of malarial parasites due to their favorable climate and increased number of vector-mosquitoes.[20,21] Another study from Saudi Arabia observed 0.0% positivity rate for malarial antigens in endemic and non-endemic regions.[22,23] However, actual prevalence could be high because both antigen detection tests or microscopy cannot detect low parasite density. A study from Eastern China reported presence of malarial antibodies in 2.13% of blood donors.[24] In Malaysia, 33.6% prevalence of malaria was detected which is in disagreement with the current study.[25] Molecular screening was carried out on 400 healthy blood donors in Egypt to detect ssrRNA gene of *Plasmodium* but no subclinical malarial infection was detected using PCR which is comparable with the current study. This was attributed to implementation of effective donor selection criteria as recommended by World Health Organization (WHO).[26]

Donor screening serves as a fundamental step in enhancing safety and quality of blood and blood products thereby ensuring the deferral of unhealthy donors.[14] The current study highlights the proper usage of structured questionnaire along with verbal screening based on identification of malaria risk factors in regions with low endemicity. Moreover, staff should be trained and vigilant enough to recognize and explain the questions to donor if they found any difficulty in understanding due to low literacy levels, which is a well-recognized issue in developing countries. This is also economical because donors are deferred at this stage which subsequently reduces the wastage of resources such as consumables, screening tests and saves donor and staff time. Malaria infected donor was lost to follow up so in our opinion, it might be possible that donor did not comprehend the questions or deliberately provided inaccurate information owing to fear of deferral. Hence, absence of information regarding previous exposure or malaria risk factors was noted upon retrieval of donor questionnaire. The present investigation also revealed that only male blood donors attended the transfusion center. This could be attributed to social factors associated with female donation followed by deferral due to anemia, lactation or pregnancy which encapsulated the exclusion criteria. The PCR-based detection of plasmodial DNA has been suggested as a method of choice in endemic countries. In developing countries like Pakistan, the high cost, expensive infrastructure and laboratory personnel expertise hinders wide spread utilization of molecular assays. However, efforts should be made to implement NAT-based detection in regions with high endemicity considering low

parasitemia. As epidemiology changes, the questionnaire should be reviewed periodically.

Conclusion

Malaria appears to be very uncommon in the blood of asymptomatic male donors and screening by expensive NAT would not make economic sense. Effective donor screening using structured questionnaire to defer high risk individuals is necessitated.

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