Occurrence of Transfusion Transmitted Malaria Parasites among Voluntary Blood Donors Using CareStart™ Cassette and Quantitative Buffy Coat Techniques at Kenyatta National Hospital, Kenya

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Abstract

Background: Malaria is a killer disease which can be transmitted through transfusion of blood and blood products such as packed red blood cells, fresh frozen plasma, platelets concentrate and cryoprecipitate. In medical setups, blood and its products are screened for other transfusion transmitted infections but not for malaria parasites. Recipient of blood and blood products are given antimalarial post transfusion.

Specific Objectives: The specific objectives of this study were to determine the prevalence of malaria parasites among voluntary blood donors using Quantitative Buffy Coat, malaria Carestart™ cassette and also to determine the level of agreement between quantitative Buffy coat and malaria Carestart™ cassette diagnostic techniques. Microscopic technique was used as the gold standard.

Design: The study applied across sectional descriptive study design.

Subjects: The study involved recruitment of 155 voluntary blood donors at Kenyatta National Hospital. The blood samples were subjected to three malaria screening techniques which included microscopy, quantitative buffy coat and malaria Carestart™ cassette.

Method: After blood collection from the blood donors, samples were screened for presence or absence of malaria parasites using malaria Carestart™ cassette and Quantitative Buffy Coat techniques and microscopy which was used as the gold standard.

Results: The study revealed that the prevalence of malaria infection among voluntary donors by microscopy was 5/155 (3.2%), Quantitative buffy coat was 6/155 (3.9%) and malaria Carestart™ cassette technique was 8/155 (5.2%).

Conclusion: This study confirmed that the prevalence of Malaria parasites among blood donors that are seen at Kenyatta National Hospital was 3.2% by microscopic technique, quantitative Buffy coat 3.9% and Malaria Carestart TM Cassette technique 5.2%. This implied that blood donations contained some level of malaria parasites which is a healthy risk to patients under transfusion.

Keywords: Transfusion transmitted malaria parasites, Voluntary blood donors, Quantitative Buffy Coat, Blood products, Fluorescing parasites.
Introduction
Malaria is a mosquito borne disease which is prevalent in the tropical and subtropical regions of the world. The disease is mainly transmitted through the bite of an infected female *Anopheles* mosquito carrying the infective stage which is the sporozoite. Transmission can also occur through transfusion of infected blood and its products. A recent study demonstrated relatively high likelihood of TTM via transfusion insub-Saharan African countries, illustrated by a median prevalence of malaria and determined by the evaluation of thick smears of 10.2 percent (range 0.7 percent in Kenya to 55 percent in Nigeria) in donor blood samples (Owusu-Ofori et al., 2013). Guiguemide (1992) reported that, in Burkina Faso about 14 percent of donors were infected with *P. falciparum* and higher densities and infectivity of malaria parasites among the silent carriers under 55 years old.

A study carried out by Ali, Gader, Kadaru, & Mustafa (2004) in Sudan established that the prevalence of infected donors was 6.5%, the majority of them were between 20 and 40 years old. In endemic countries, distinguishing cases of TTM from natural infections is quite a challenging task, as malaria, occurring post transfusion, can be either as a result of a natural infection or transfusion. Thus, the incidence of TTM in malaria endemic countries is disputably under reported (Brouwer et al., 2013). The current study was done to determine Prevalence of Transfusion Transmitted Malaria Parasite Among Blood Donors Using Carestart™ Cassette and Quantitative Buffy Coat techniques. The purpose of writing this paper was to advice policy makers in hospital setups that there is a need to develop comprehensive national policies for safe blood and its products administration to recipients.

Materials and Methods
The permission to conduct the current study was obtained from Kenyatta National Hospital – University of Nairobi Ethics and Research Committee (KNH-UoN ERC) and the reference number was KNH-ERC/A/340. The study involved recruitment of 155 voluntary blood donors at Kenyatta National Hospital between the month of March and May 2017. The study participants gave informed consent by signing a written consent form. Sample collection procedures and processing proceeded.

Test Methods
Microscopic Technique: A thick film was made by placing 6µl of donor’s blood at the Centre of grease-free microscopic slide. Without delay, the blood was spread with a glass spreader held at a steep angle to achieve a thick smear covering an area of 15 x 15 mm. This was allowed to air dry. Then 10% Giemsastain was applied on the thick film and allowed for 20 minutes. After this time, the stain was washed off using distilled water and again allowed to air-dry. The dried stained thick smear was viewed under a light microscope using 100x oil immersion objective lens. Results were observed and recorded.

Malaria Carestart™ Cassette Technique
Five µl of blood was added on the sample well labeled S. Two drops of buffer were added on the buffer well labeled A. Results were taken after 20 minutes.

Quantitative Buffy Coat Technique
The tube was filled with 60 microliters of blood. A clear plastic closure was then attached. A precisely made cylindrical float, designed to be suspended in the packed red blood cells, was inserted. The tube was centrifuged at 12,000 rpm for 5 minutes. The components of the buffy coat were separated according to their densities, forming discrete bands. The QBC tube was placed on the tube holder and examined using a standard white light microscope equipped with the UV microscope adapter, using X60 microscope objective. Fluorescing parasites were observed at the red blood cell or white blood cell interface (Buffy Coat). A negative test was reported within one minute and positive result within minutes.
Results
The prevalence of malaria parasites among blood donors was determined using Quantitative Buffy Coat Technique and Malaria Carestart™ Cassette Technique. Microscopic technique was used as the gold standard.

Demographic Data Analysis
This cross sectional study was conducted in Kenyatta National Hospital Kenya on 155 voluntary replacement blood donors who presented them in the study without any sign of illness between March to May 2017.

Table 1: Donors screened for malaria parasites with respect to age and sex

| Age  | Males Screened % | Females screened % |
|------|------------------|--------------------|
| 10-19| 4.7(5)           | 6.3(3)             |
| 20-29| 62.6(67)         | 52.1(25)           |
| 30-39| 26.2(28)         | 35.4(17)           |
| 40-49| 6.5(7)           | 4.2(2)             |
| 50-59| 0.0(0)           | 1(1)               |
| Total|                  | 100                |

Table 2: Donors tested positive for Malaria Parasites using microscopy with respect to age and sex.

| Age  | Males Positive % | Females Positive % |
|------|------------------|--------------------|
| 10-19| 0.0              | 0.0                |
| 20-29| 0.0              | 0.0                |
| 30-39| 4.7(5)           | 0.0                |
| 40-49| 0.0              | 0.0                |
| 50-59| 0.0              | 0.0                |
| Total| 4.7              | 0.0                |

Table 3: Prevalence of malaria parasites among blood donors.

| Technique                  | Samples Tested Positive for malaria parasites | Prevalence (%) |
|----------------------------|-----------------------------------------------|----------------|
| Microscopy (Goldstandard)  | 5                                             | 3.2            |
| Rapid Diagnostic Test      | 8                                             | 5.2            |
| Quantitative Buffy Coat Technique | 6                           | 3.9            |

The point prevalence of malaria parasites among blood donors was determined using rapid diagnostic technique and quantitative buffy coat and they were compared against microscopy. Microscopy technique reported a prevalence of 3.2%, Carestart™ Cassette technique 5.2% and quantitative buffy coat reported prevalence of 3.9 % as shown on the table 3 above. The prevalence of blood donors who were infected by malaria parasites using the three techniques was arrived as show below. Microscopy-blood donors truly affected by malaria parasites were 5, population of blood donors were 155 then multiplied by 100. Malaria Carestart™ Cassette, blood donors truly affected by malaria parasites were 8, population of blood donors were 155 then multiplied by 100. Quantitative Buffy Coat technique-blood donors truly affected by malaria parasites were 8, population of blood donors was 155 then multiplied by 100.

Discussion
This present study aimed at determining the prevalence of malaria parasites among blood donors using Carestart™ Cassette and quantitative buffy coat techniques against microscopy. The transmission of malaria infection through blood transfusion is one of the oldest recorded incidents of transfusion transmitted infections in transfusion facilities due to lack of screening. Malaria transmissions associated with blood transfusion in malaria prone zones has been on the rise. This study has confirmed that blood donors that are seen at Kenyatta National Hospital have a prevalence of 3.2%. Based on observations from malaria screening questionnaire directed to a symptomatology of blood donors; this study has showed that voluntary blood donors contribute to the risk of transfusion transmitted malaria in low and high prevalence zones. While Nairobi is classified as low malaria endemic zone transmission of malaria through blood transfusion is still present. A study by Kitchen et al., (2005) showed that transmission of malaria infection by blood transfusion is a possible cause of malaria infections among blood donors and it agrees with this study. Other similar studies carried out including a study carried out in Abakaliki Metropolis obtained a prevalence of 40.9% among blood donors which was high contrary to this study (Epidi et al., 2008). The difference in those
studies and this study could have been caused by differences in sample size used, endemicity, period during which the data was collected and the technique used for diagnosis. While a lower malaria prevalence of 1% was reported in Ethiopia among blood donors this study was comparable to other studies that were done in Ghana (10%), Sudan (5%) and Yaoundé (6.5%) (M. S. M. Ali et al., 2004; Noubouossie et al., 2012; Owusu-Ofori et al., 2013).

In this study, the prevalence of malaria infection among blood donors was found to be 3.2% by microscopy, Quantitative Buffy Coat 3.9 % while the Carestart™ Cassette technique gave a prevalence of 5.2 %. Quantitative Buffy coat technique results compared well with microscopy because it picked all positives picked by the gold standard. Carestart™ Cassette technique yielded higher prevalence values above the gold standard because it picked more false positive values than microscopy. Carestart™ Cassette technique was found to detect more false positives than QBC and microscopy. This means that Carestart™ Cassette technique is more prone to false positives than the gold standard. This could have been attributed by confounders such drug resistance, persistence of antigens due to sequestration, incomplete malaria treatment, cross reaction with autoantibodies such as rheumatoid factor and presence of histidine rich protein 2 antigen of plasmodium parasite in the blood of the donor gene deletion or mutation of Histidine Rich Protein-2 (HRP-2) as postulated by Wellems, Walker-Jonah & Panton, (1991) and agreed with a study done by Latha,(2016). The Carestart™ Cassette technique was less accurate in determination of malaria prevalence at KNH compared to the QBC.

The prevalence of malaria parasite infection in blood donor units using QBC and Carestart™ Cassette technique against microscopy obtained in this study was low, although not insignificant. This low prevalence may be accounted for by the study period which was a low and rainy season and there was no much travelling of people from plasmodium endemic region, hence low transmission rates of malaria. The timing of the study from March to May, and the duration of three months, did not cover the peak of long rainfall period of October to December. This timing could have influenced the prevalence of malaria in the population. Malaria transmission is favoured by the availability of stagnant surface waters that are abundant during the rainy season; these act as breeding sites for the vector mosquitoes of the infection. Another factor influencing the study's low donor prevalence could be the relatively small sample size of 155, although statistically it is adequate to demonstrate the desired parameter.

However, a larger sample size would have increased the power or accuracy of the study, and the obtained prevalence rate might have been higher. This effect of sample size can be seen in the studies from South-Eastern Nigeria that had large sample sizes and concomitant high prevalence of 40.9% and 30.2% respectively (Okocha et al., 2005; Uneke, Ogbu, & Nwojjji, 2006). On the other hand, the studies from Jos (Ikeh & Okeke, 2006) and Zaria (Garba et al., 2009) with small sample sizes had lower prevalence of 11% and 6.8%, respectively. In another similar study which was done by Douglas D et al., (2016) in Nigeria the prevalence of malaria parasites among blood donors was 7.5% which was also relatively low.

Conclusion
This study confirmed that the prevalence of Malaria parasites among blood donors that are seen at Kenyatta National Hospital was 3.2% by microscopic technique, quantitative buffy coat 3.9% and rapid diagnostic test 5.2%. This implied that blood donations contained some level of malaria parasites which is a healthy risk to patients under transfusion.

Recommendations
1) All blood donations are subjects of infection with malaria and as a result, all
blood should be screened for malaria parasites before transfusing the recipients.

2) In a view of this research finding there is a need to develop comprehensive national policies for safe blood and its products administration to recipients.

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