Prevalence of malaria parasitaemia and methaemoglobin levels among blood donors in Sokoto, Nigeria

Isah Zama¹, Abdulrahman Yakubu¹, Augustine N. Okwesili¹, EY Ishaku¹, Osaro Erhabor^{1*}, Abdullah S. Mainasara² and Kufre E. Uko³

*Correspondence: n_osaro@yahoo.com

¹Department of Haematology, Faculty of Medical Laboratory Science, Usmanu Danfodiyo University, Sokoto, Nigeria.

²Department of Biochemistry, Faculty of Medical Laboratory Science, Usmanu Danfodiyo University, Sokoto, Nigeria.

³Department of Haematology, University of Calabar, Nigeria.

Abstract

Background: Safety of blood and blood products is of global concern in transfusion medicine especially as it concerns the transfusion transmissible malaria infection scourging the tropics. Malaria parasite also disrupts haemoglobin pigment converting haemoglobin to non functional methaemoglobin. This study was taken to determine the prevalence of malaria parasitaemia and methaemoglobin level among blood donors attending Usmanu Danfodiyo University Teaching Hospital Sokoto (UDUTH).

Method: Two hundred and twenty eight (228) consecutively-recruited apparently healthy male blood donors aged 18–45 years were tested for methaemoglobin level using the modified Evelyn and Malloy method. Malaria testing was done using thin films made by push wedge technique prepared from the EDTA-anticoagulated blood was stained with Giemsa stain. Parasite counts were reported per 500 white blood cells (WBC). The total parasite count was determined and result expressed as the number of parasites per microlitre of blood.

Result: Among the 228 blood donors screened, 74 of the subjects representing 32.5% were positive for Malaria and 154 representing 67.5% tested negative. *Plasmodium falciparum* was responsible for all cases of parasitaemia. The mean parasite load among the parasitized donors was 228 ± 99 parasites per microliter of blood. Among the malaria-infected donors, 60 (67.5%) had a parasite density of $\leq 500 \mu\text{L}$, while 14 (6.2%) had a parasite density of $501\text{--}10000 \mu\text{L}$. The mean methaemoglobin levels among malaria infected donors was significantly higher ($p=0.002$) among plasmodium parasitized (2.75% and 3.55%) compared to non-parasitized donors (2.0%). We observed a significant positive correlation between parasite density and methaemoglobin level ($r=0.72$, $p=0.001$).

Conclusion: This present study indicates a high prevalence of malaria among the blood donors studied. It may be justifiable for recipients of blood transfusion particularly neonates, children and pregnant women in malaria-endemic environment to be routinely treated with antimalarial drugs as a prophylactic measure. We advocate for a mandatory universal donor-screening policy for malaria and for the exclusion of blood donors with malaria parasitaemia to further enhance blood safety in our environment.

Keywords: Malaria parasite, methaemoglobin levels, blood donors, Sokoto, Nigeria

Introduction

Malaria is one of the world's deadliest diseases affecting people particularly in tropical and sub-tropical regions of the world. Malaria remains the most complex and overwhelming health problem facing humanity [1], with 300 to 500 million cases and 2 to 3 million deaths per year [2]. The disease imposes serious effect on the blood, destroying red blood cells and interfering with the haemoglobin, disrupting the red blood cells pigment and converting haemoglobin to methaemoglobin leading to methaemoglobinaemia. Malaria was once widespread in North America and other temperate regions. Today, the disease occurs mostly in tropical and subtropical regions, particularly in sub-Saharan Africa and Southeast Asia [3]. About 90% of all malaria infections in the world today occur in Africa South of the Sahara. Majority of infections in the region are caused by *Plasmodium falciparum*, the most dangerous and the most effective malaria vector of the four human malaria parasites [2].

The administration of blood to a patient is potentially a life saving procedure and the demand for blood has greatly increased over the years [4]. Transfusion therapy although

a very important form of treatment in humans, it may be the only option for survival for many patients. Although this therapy helps to save lives, blood can nonetheless be a vehicle for transmission of infections including parasitic diseases [1].

Malaria infection has become of more interest to blood banking and blood transfusion based on discoveries that malaria infection may cause methaemoglobinaemia, as haemoglobin taken up by the parasites into their acid food vacuole leads to the spontaneous oxidation of ferrous (Fe^{2+}) to ferric (Fe^{3+}) iron. Methaemoglobin is a dysfunctional form of hemoglobin that is incapable of transporting oxygen. Wright defined methaemoglobinemia as an altered state of haemoglobin in which the ferrous form of haem is oxidized to the ferric form thus making the haem moiety unable to bind oxygen [5]. In addition, the remaining monomers of ferrous haem within a haemoglobin tetramer bind their oxygen more tightly causing a left shift of the oxygen dissociation curve and reduced oxygen delivery at the tissue level. Methaemoglobin is formed when the haem part of haemoglobin is oxidized by accepting an electron from free radicals in the blood. In

healthy subjects, blood methaemoglobin levels are low, typically < 2% of the total haemoglobin in the blood. When Methaemoglobin concentrations are increased (a condition called methaemoglobinemia), there is less available functional haemoglobin to carry oxygen for systemic delivery [6] leading to varying complications from skin discolouration, cyanosis, weakness, confusion seizures and death.

In every country, surgery, trauma, severe anaemia and complications of pregnancy are among the clinical conditions that demand blood transfusion, with a much greater proportion of transfusions being given to women with obstetric emergencies and children suffering from severe anaemia, often resulting from malaria and malnutrition. Blood transfusion is an important tool in the management and treatment of anaemia and when administered helps to improve the oxygen carrying capacity of blood. In Nigeria, screening for malaria parasite is neither routinely done in blood banks, nor stipulated in the current National Blood Transfusion guidelines. This is because transmission of malaria through blood transfusion is generally not regarded as a serious problem, in adult and adolescent whose level of immunity is thought to be sufficiently effective in combating post transfusion malaria in malaria endemic areas [7].

Commercial and family replacement donation is prevalent in Nigeria. The World Health Organization advocates that blood donation should be voluntary and non-remunerated. Commercially remunerated donors tends to come from the poorest sectors of society, are more likely to live in densely populated, malaria-infested, poor-sanitary environments, more likely to be poor in health, under-nourished, more likely to give blood more often than is recommended and are more likely to transmit transfusion-transmissible infections [8]. Nigeria like many other tropical developing countries have a high level of occurrence of blood-demanding health conditions due to increase in road traffic accidents, pregnancy-related haemorrhage, armed robbery attacks, communal clashes and violent events, has amplified the possibility of the transmission of blood-borne diseases. This implies that blood transfused to anaemic patients may be positive for malaria parasites and can contain a high percentage of methaemoglobin from the possible oxidative metabolic action of *plasmodium* on haemoglobin, resulting in the desired aim of blood transfusion to correct anaemia and increase the oxygen carrying capacity of the recipient blood not met. It is against this background that this study aims to determine the prevalence of malaria parasitaemia and methaemoglobin levels among blood donors in UDUTH, Sokoto State.

Methods

Study site

This present study was carried out at the Haematology and Blood Transfusion Department of Usmanu Danfodiyo University Teaching Hospital (UDUTH) in Sokoto Nigeria. The hospital is a tertiary health institution located in Sokoto metropolis

committed to the provision of quality tertiary healthcare services to the entire North-western region and neighboring border country - Niger Republic. The state is located between longitudes 11° 30 to 13° 50 East and latitude 4° to 6° North. It has a land area of about 28,232.37sq kilometer and stands at an altitude of 272 m above sea level near to the confluence of the Sokoto River and the Rima River. Sokoto state is at the extreme Northwest of Nigeria forming a border with Niger Republic. The state is in the dry Sahel surrounded by sandy terrain and isolated hills with an average annual temperature of 28.3°C (82.9°F). The weather is characterized by two seasons the wet and dry seasons. Rainfall (wet season) starts late around June and ends in September sometimes extending into October. The average annual rainfall is 550 mm with peak rainfall usually recorded in the month of August. The highest temperatures of 45°C during the hot season are experienced in the months of March and April. Harmattan, a dry cold and dusty condition is experienced between the months of November and February. Sokoto state had a population of 4.2million as at the 2006 census. The metropolis is estimated to have a population of 427,760 people [9] made up of Hausa and Fulani majority and a minority of Zabarmawa and Tuareg and other non-indigenous settlers. The two major languages in the state is Hausa and Fulfulde is spoken among the Fulani. The main occupation of the people is grain production and animal husbandry. Majority of the indigenous people practice agriculture. Crops produced include commercial crops like millet, sorghum, beans, rice and maize. Other occupations commonly practiced are dying, blacksmithing, weaving, carving, trading, and cobbling. Sokoto ranks second in livestock production in Nigeria. Modern Sokoto city is a major commercial center in leather crafts and agricultural products. Occupation of city inhabitants also include trading, commerce, with a reasonable proportion of the population working in private and public sectors [10]. Socio cultural characteristics is homogenous as majority of its indigenes and inhabitants are Muslims, therefore the doctrines of Islam provides the singular code of conduct and behavioral characteristics generally accepted across the State. Common practices are early marriage, polygamy, consanguinity and multiple births.

Study participants

The subjects for this study included two hundred and twenty eight (228) consecutively-recruited apparently healthy male blood donors aged 18 – 45 years visiting the Haematology and Blood Transfusion unit for blood donation purposes. Only donors who met the inclusion criteria of age and blood donation requirement of haemoglobin ($\geq 12.5\text{g/dl}$) and those who gave informed consent after counselling were enrolled into the study.

Laboratory investigations

About five milliliters of blood of whole venous blood was collected into EDTA anticoagulated tubes. Modified Evelyn

and Malloy Method [11] was used for the estimation of methaemoglobin level following strictly the manufacturer's standard operating procedures. The absorbance of methaemoglobin in erythrocyte lysate was measured with a spectrophotometer at a specific wavelength of 630nm (S1). In summary, potassium cyanide (KCN) was added to red cell lysate to facilitate the conversion of methaemoglobin to cyanmethaemoglobin, which does not absorb at 630nm (S2), hence, the difference between absorbance readings S1 and S2 represents the absorbance due to methaemoglobin. On addition of potassium ferricyanide ($K_3Fe(CN)_6$), all forms of haemoglobin are converted to methaemoglobin the absorbance at 630nm was recorded (T1). KCN was subsequently added and the absorbance recorded (T2). The percentage of methaemoglobin in the sample was calculated as $[100(S1-S2)] / [10(T1-T2)]$.

Malaria testing was done using thin films prepared from the EDTA-anticoagulated blood and stained with Giemsa stain. The thin film was made by the push wedge technique. Parasite counts were reported per 500 white blood cells (WBC). The total parasite count was determined and result expressed as the number of parasites per microlitre of blood. The smears were examined using 100x oil immersion. A well-stained area, free of precipitates and well-populated with white blood cells (10-20 WBCs/ field) was selected. No Parasite Found (NPF) was reported after 100 fields, each containing approximately 20 WBCs. These smears were examined to determine the presence of malaria parasites, calculation of the parasite load and speciation of the malaria parasites. Examination was done using 100 x oil immersion objective.

Estimation of parasite density

Malaria parasite densities were recorded as a ratio of parasites to white blood cells (WBCs) from the thin smears. To quantify malaria parasites against WBCs on the thin smear, the parasites were tallied against WBCs until 500 WBCs were counted. Densities (parasite per microlitre of whole blood) were then calculated as follows:

Parasites/microlitre blood = parasites/WBC x WBC count per microlitre.

For the subjects in this study, parasite densities were calculated as follows:

Parasites/500 x WBC count of individual blood sample.

Plasmodium parasitaemia was graded as follows: $\leq 500 \mu/L$ = (+); $501 \mu/L - 10000 \mu/L$ = (++) and $\geq 10,001 \mu/L$ = (+++).

Statistical analysis

Statistical analyses were conducted using SPSS (version 11) software. Comparisons between populations were made using the Student's t-test for parametric data and the Mann-Whitney test for non-parametric data. An alpha value of < 0.05 denoted a statistically significant difference. Correlation was compared using a version of linear regression analysis.

Results

As shown in (Figure 1) below a total of 228 blood donors screened for malaria 74 of the subjects representing 32.5% were positive for Malaria and 154 representing 67.5% of screened donors tested negative. *Plasmodium falciparum* was responsible for all cases of parasitaemia. The mean parasite load among the plasmodium-parasitized donors was 228 ± 99 parasite per microliter of blood. Among the malaria-infected donors, 60 (67.5%) had a parasite density of $\leq 500 \mu/L$, while 14 (6.2%) had a parasite density of $501 \mu/L - 10000 \mu/L$.

The mean methaemoglobin concentration was significantly higher among malaria parasitized donors (2.75% and 3.55%) respectively with parasite density of $\leq 500 \mu/L$ and $501 \mu/L - 10000 \mu/L$ compared to non-infected blood donors (2.0%). The mean methaemoglobin levels was significantly higher ($p=0.002$) among malaria infected donors with parasite density of $\leq 500 \mu/L$ and $501 \mu/L - 10000 \mu/L$ (2.75% and 3.55%) compared to non-infected blood donors (2.0%). We observed a significant positive correlation between parasite density and methaemoglobin level ($r=0.72$, $p=0.001$). (Table 1) show the methaemoglobin level among blood donor tested (Figure 2).

Table 1. Methaemoglobin levels in relation to the degree of Parasitaemia among Blood Donors.

Level of parasitaemia	Number of subjects	Methaemoglobin level (%)	P-value
Negative	154	2.0	0.002
≤500µ/L	60	2.75	
501 µ/L – 10000 µ/L	14	3.55	

Discussion

Malaria is one of the most widespread infections globally and is a major cause of mortality, particularly in regions of high malaria endemicity. In this study carried out between October to December, we investigated the prevalence of transfusion-transmissible malaria infection and methaemoglobin level among donors in Sokoto Nigeria. In this present study, we observed a malaria prevalence of 32.5% among the donors tested. Blood donor testing for malaria parasitaemia is not routinely done in the transfusion laboratory in Sokoto Nigeria. This could mean that a relatively high probability of malaria transmission through blood transfusion of asymptomatic donor units. Our findings is higher than prevalence of 28% and 10.2% respectively observed by Agboola and co-workers [4] in Lagos University Teaching Hospital (LUTH) and by Erhabor *et al.*, in Port Harcourt Nigeria [8]. Other similar studies carried out around the country including a study carried out in the South East of Nigeria showed a prevalence rate of 40.9% [12]. In Abakaliki metropolis, Epidi *et al.*, [13] in their work obtained malaria prevalence of (51.5%) among their blood donors. Similarly Ekwunife [1] reported an alarming high rate (74.1%) of malaria infection among blood donors in Onitsha urban area. The very high prevalence rates recorded by various researchers in the South Eastern part of the country as compared to the 32.5% prevalence obtained in this present study in Sokoto may be largely be due to difference in geographical zone. Constant minimum temperatures of 16–18 8C (optimum: 20–30°C) and high humidity for several weeks are pre-conditions for vectoral transmission of malaria [14]. The vegetation characteristic in the South is that of the tropical rain forest with an average annual rainfall of about 1600mm and a high atmospheric temperature around the year. This provides available surface water and pools for the breeding of the disease vector and subsequently the spread of the disease. Whereas the weather in the Sahel savannah of Sokoto only encourages the spread of the disease particularly during rainy season which starts late in June and ends early in September but may sometimes extend into October. The average annual rainfall is 550 mm with peak in the month August. Our finding is also consistent with report by Abdullahi *et al.*, [15] who obtained a malaria prevalence of 23.4% in Sokoto metropolis in their study carried out between the months of October to December.

In this study, we observed that there was no relationship between the presence of malaria parasitaemia and clinical malaria among donors in Sokoto where current measures to prevent transfusion-transmissible malaria depend mainly

on donor selection using questionnaires in which potential donors are only deferred based on their specific answers during the donor-screening process. A number of recent cases of transfusion-transmitted malaria have been attributed to failure of the questioning process itself or to an unexpectedly long incubation periods of *Plasmodium falciparum*. The high prevalence of self-medication in our environment may result in sub-optimal treatment of malaria infection and may enable the persistence of malaria parasites in an infected donor's blood. However, the argument against universal screening of blood donors for malaria, particularly, in non-endemic countries is that the risk of transfusion-transmitted malaria is minimal and the incidence of malaria in the general population is low. Universal screening of donors for malaria, particularly, in high-endemicity regions in sub-Saharan Africa will further enhance blood transfusion safety. Although several methods of malaria diagnosis in donors have been developed, they are either too sensitive or unable to detect all types of plasmodial infection. The only test with adequate sensitivity and specificity is an immunofluorescence assay. This assay may be unavailable in resource-poor malaria- endemic countries. In the midst of these challenges, we advocate that malaria diagnosis using thick and thin blood film stained with Giemsa could become an inexpensive, cost effective and readily available minimal alternative, particularly, in resource-limited, malaria-endemic settings in sub-Saharan Africa to protect the integrity and purity of blood supply.

Consistent with previous reports [8,16,17,19], we found *P. falciparum* the predominant species among plasmodium-parasitized donors. *Plasmodium falciparum* malaria may be associated with a potentially fatal outcome, particularly if there are delays in recognition and treatment [15]. However, there is increasing advocacy for recipients of blood transfusion particularly neonates, children and pregnant women in malaria-endemic areas to be routinely treated with antimalarial drugs as a prophylactic measure. We advocate for a mandatory universal donor-screening policy for malaria, for the exclusion of blood donors with plasmodial parasitaemia and for discouraging remunerative and family -replacement blood donation and for setting up a national blood transfusion service operated on voluntary non-remunerated low- risk blood given out of altruism to further enhance blood safety.

Transfusion malaria was described as particularly common in countries where blood donation has become a commercial transaction and where the blood donors come from less affluent social classes [19].

In this present study, the mean parasite load observed among asymptomatic plasmodium parasitized donors was 228 ± 99 parasite per microliter of blood. The parasite load is much lower than a mean parasite count of 2650 ± 234 parasites/µl observed in a previous study [20] among pregnant women with active/symptomatic case of malaria infection. A previous report [8] from Nigeria, which investigated the prevalence of malaria among blood donors, observed that

there was no relationship between the presence of malaria parasitaemia and clinical malaria. Current measures in the study environment, to prevent transfusion-transmissible malaria depend mainly on donor selection using questionnaires. Potential donors are only deferred based on their specific answers during the donor-screening process. A number of recent cases of transfusion malaria have been attributed to failure of the questioning process itself or to an unexpectedly long incubation periods of *Plasmodium falciparum*. There may be need to introduce universal screening of blood donors for malaria particularly in malaria endemic countries. However, the argument against universal screening of blood donors for malaria, particularly, in non-endemic countries is that the risk of transfusion-transmitted malaria is minimal and the incidence of malaria in the general population is low. Universal screening of donors for malaria, particularly, in high-endemicity regions in sub-Saharan Africa will further enhance blood transfusion safety.

In this present study, the concentration of methaemoglobin was significantly higher among plasmodium parasitized compared to non-parasitized donors. We observed a significant and positive correlation between the level of parasitaemia and methaemoglobin level among parasitized donors. Our finding is consistent with the result obtained by Uko and co-workers [21] among malaria infected children in Calabar which showed that patients with severe malaria parasitaemia had markedly raised methaemoglobin values compared to those with mild/moderate malaria infection. Our result in this present study shows that there is relatively high prevalence of malaria infection among blood donors in Sokoto, Nigeria and that the level of methaemoglobin concentration is significantly higher among malaria parasitized compared to non-parasitized donors.

Limitation

This present study had several limitations. Firstly, convenience sampling was used in the recruitment of subjects. Subjects were consecutively recruited blood donors who met the inclusion criteria of age and informed consent. This sampling method may have introduced the possibility of selection bias. Secondly, it is possible that seasonal variation may have affected the study findings. Previous studies [15,22,23] indicates that malaria infestation in any population could vary by seasons and is particularly higher during the raining season.

Recommendations

This present study indicates a high prevalence of malaria among blood donors studied. It may be justifiable for recipients of blood transfusion particularly neonates, children and pregnant women in our malaria-endemic environment to be routinely treated with antimalarial drugs as a prophylactic measure. We advocate for a mandatory universal donor-screening policy for malaria, for exclusion of blood donors with plasmodia parasitaemia to further enhance blood safety

in our environment.

Competing interests

The authors declares that they have no competing interests.

Authors' contributions

Isah IZ and Yakubu A designed the study, Okwesili AN and Ishaku EY recruited the subjects and carried out the laboratory testing while Erhabor O, Mainasara AS and Uko EK were involved in the statistical analysis and writing up the manuscript.

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