Association of Asymptomatic Malaria and ABO Blood Group among Donors Attending Asamankese Government Hospital

David N Ofosu1,2, Charles Dotsey1, Yeboah M Debreyeli1

1Department of Medical Laboratory, Garden City University College
2Department of Medical Laboratory Technology, Faculty of Allied Health Science, Kwame Nkrumah University of Science and Technology

Abstract: Background: Transmission of malaria by blood transfusion remains a significant public health problem in the malaria endemic regions like Ghana. Consequently, a high risk of transfusion-transmitted malaria persists. Blood donors are not routinely screened for malaria parasites prior to donation, therefore the risk of transmission of malaria parasites to blood recipients and the likelihood of their development of clinical disease remains high. This study aimed to find out the frequency of malaria in apparently healthy blood donors of Asamankese Government Hospital and its association with ABO and Rh blood groups. Methodology: Cross-sectional study was conducted involving 323 blood donors at the Asamankese Government Hospital. Donor blood samples collected were analyzed for the detection of malaria parasites and ABO/Rh blood group. Questionnaire was used to gather socio-demographic characteristics. Results: The most prevalent population of blood donors were bloodgroup O and rhesus positive with 58.2% and 91.0% respectively. About 45.8% were paid donors. The prevalence of asymptomatic malaria in the population was 2.8%. Rhesus positive blood group was significantly associated with decreased risk of asymptomatic malaria infection (OR = 0.2, P = 0.020). Blood groups A and AB were significantly associated with malaria infection (OR = 1.8, 15 and P = 0.432 and 0.030 respectively). Conclusion: Study demonstrated a high prevalence of malaria infection among blood donors of Asamankese Government hospital with majority being paid donors. Blood group O and rhesus positive are protective against asymptomatic malaria infection.

Keywords: Malaria, blood donors, blood group, transfusion

1. Introduction

Malaria is a very important disease in Sub-Saharan Africa with high morbidity and mortality rates (Muntaka and Opoku-Okrarah, 2013; Owusu-Ofori et al., 2013) and recent estimates from World Health Organization (WHO) show that there were 214 million new cases of malaria worldwide in the year 2015.

Plasmodium recognition in donor blood by microscopy has only been instigated in a few regions but is inefficient. Consequently, a high risk of transfusion-transmitted malaria persists. Because of resource restrictions, the most common red blood cell product transfused is whole blood, and it is often transfused within a week of collection. (Mogtomo et al., 2016). Thirty percent (30%) of blood donors of Nigeria and of Benin are malaria infected and paradoxically, the biological screening of blood viral infection is performed in several African blood banks, but that of malaria barely done. (Tagny et al., 2008)

The incidence of parasitaemia in blood donors and patients in Ghana was around 50%, and the actual incidence of transmission in non-parasitaemia blood recipients transfused with parasitemic whole blood was about 14–28% (Mogtomo et al 2016), while (Owusu-Ofori et al., 2013) also reported the prevalence of malaria parasitaemia in blood donors varying from 0.6% to 50% in sub-Saharan Africa.

Blood donors are not routinely screened for malaria parasites prior to donation, therefore there is the risk of transmission of malaria parasites to blood recipients and the likelihood of their development of clinical disease (Wariso and Oboro, 2015b). The malaria parasite can survive for 3 weeks or more in refrigerated blood. The risk of transmission is even higher in cases of transfusion of fresh whole blood, particularly when the blood has been stored for less than 5 days with the risk being reduced considerably after 2 weeks. (Wariso and Oboro, 2015a). Transfusion Transmitted Malaria (TTM), compared to natural infection often has a short incubation period because there is no pre-erythrocytic development. This also depends on the species of parasite introduced which varies from 10 days in P. falciparum to 40 days or longer in P. malariae (Sulzer et al., 1978; Lakshmi and Anuradha, 2015).

Transfusion therapy is the use of blood and blood products as a form of treatment to save lives. However, this process can lead to transmission transmitted infections such as malaria parasites if blood is received from a malaria infected donor (Muntaka and Opoku-Okrarah, 2013). An initial report on malaria as a result of blood transfusion has been published in 1911. Evidence shows that of all the four human parasites of malaria, Plasmodium falciparum and Plasmodium Malariae are the most prevalent while P. falciparum infection is the most severe. (Muntaka and Opoku-Okrarah, 2013; Grant et al., 2015; Organization, 2015)

It is hypothesized that Plasmodium falciparum malaria has shaped the distribution of ABO blood groups in humans. (Alemu and Mama, 2016). It has been shown that there is significant association between malaria and ABO blood groups where blood group O individuals are more susceptible than other ABO blood groups. However blood group AB individuals were more affected by malaria. (Alemu and Mama, 2016)
Transfusion-transmitted malaria (TTM) was first described in 1911. The most recent publication on global incidence of TTM, based on data from 1911 to 1979, suggests that the incidence of TTM is about 145 reported cases per year, mostly confined to endemic countries. The relatively high likelihood of TTM via donor blood in sub-Saharan African countries is illustrated by a median prevalence of malaria, determined by microscopic evaluation of thick blood smears, of 10.2% (range: 0.7% in Kenya to 55% in Nigeria) in donor blood samples. In endemic countries differentiating cases of TTM from natural infections is a challenge as malaria, occurring post-transfusion, can be the result of either a natural infection or transfusion transmitted. Hence, the number of TTM in endemic countries is unquestionably under-reported (Brouwer et al., 2013). According to (Owusu-Ofori et al., 2013) the prevalence of P. falciparum malaria in transfused blood was 4.7% (21/445) by microscopy, 13.7% (60/440) by rapid diagnostic test, a research conducted in Komfo Anokye Teaching Hospital.

The ABO polymorphism is the most recognized and the most clinically important antigen classification system to date. Its recognition is central to the practice of transfusion medicine, because of the immediate recognition and rejection of major incompatible non-self-cells. In the past century since the ABO discovery, scientists have been able to identify an association between the ABO blood type and a number of infectious diseases, some of which exert genetic selection. However, the cause of the molecular and geographic diversity, along with the evolutionary basis for the origin of the ABO blood type, remains a mystery.

ABO blood group and rhesus blood group systems have proven to be the most important, for blood transfusion purposes. Discovery of ABO blood group system disclosed the way for discoveries in the field of immunohematology, blood transfusion among humans regardless of their natives, unmatched pregnancy, legal medicine, anthropology and the discovery of other blood group systems (Matouke and Bawa, 2016).

The molecular basis of the ABO blood group system was clarified in 1990. The gene encodes a glycosyltransferase, which transfers N-acetyl D-galactosamine (group A) or D-galactose (Group B) to the nonreducing ends of glycans on glycoproteins and glycolipids.

In particular, the ABO antigens regulate cellular activities proposing their impact on determining susceptibility and rigorousness of certain diseases. It has been more than four decades since association of ABO blood group and malaria was suggested. There is also a hypothesis that Plasmodium falciparum (P. falciparum) malaria has shaped the distribution of ABO blood groups in humans. Although this therapy helps to save human lives, blood can nonetheless be a dreadful vehicle for the transmission of some infectious and parasitic diseases; among them is a malaria fever, caused by plasmodium species.

Malaria is endemic in tropical Africa and there has been some debate among transfusion practitioners in the region as to whether donor blood for transfusion to indigenous people should be screened for malaria. The consensus so far has not been reached, this situation poses a particular risk to vulnerable blood recipients,(Okocha et al., 2005)

In Ghana, 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 an endemic area like Ghana (Attah, 2000; Ekwunife et al., 2011);

Transfusion transmitted malaria, which is malaria transmitted by transfusion of blood from infected donors is one of the most common transfusion-transmissible infections. Most donors implicated in transfusion-transmitted malaria live in malarious areas and so are predominantly semi-immune with very low parasite loads and the estimated infectious dose is 1 to 10 parasites per unit of blood (Wariso and Oboro, 2015b)

The protective effect of blood group O has been attributed to smaller and easily disrupted rosette formation by blood group O (which lack A and B antigens, the receptors forrosetting on uninfected erythrocytes that bind to parasite rosetting ligands PfEMP-1 and sequestrin) compared to that formed by blood group A, B or AB erythrocytes(Wolofsky, 2009; Rout et al., 2012).

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Figure 1: Structure of ABO blood group antigen (Simiyu, 2015)

Abbreviations: R represents lipid—glucose—galactose chain, Gal, galactose; GalNAc, N-acetylgalactosamine; Fuc, fucose; GlcNAc, N-acetylglucosamine.

Relationship between ABO Blood Group System and Malaria
Numerous studies have sought to institute an association between the ABO blood types and malaria. These studies have however been unable to establish an unequivocal link between the ABO blood groups and the prevalence and occurrence of malaria parasitaemia. A study piloted at a tertiary care hospital at Navi, Mumbai, India found that people with blood group O are more prone to malarial infection in endemic areas (Singh et al., 2015).

This assertion that individuals with certain blood groups were predisposed to P. falciparum malaria infected was however not supported by a study that prevalence of malaria parasitaemia and the predisposition of the ABO blood groups to falciparum malaria among blood donors at a Ghanaian hospital (Muntaka and Opoku-Okrah, 2013).

In Ethiopia, Tekeste et al assessed 210 cases of falciparum malaria (70 severe and 140 uncomplicated) compared with 190 cases of healthy controls in the malaria endemic locations of Awash, Metehara and Ziway. Severe malaria was defined as having at least one of the severe malaria syndromes (cerebral malaria, severe anemia and circulatory collapse). Results showed that in the severe malaria category, there were 25 (35.7%), 15 (21.4%), 14 (20%) and 16 (22.9%) blood group A, B, AB and O patients, respectively. Blood group O was found to be the dominant blood type in both uncomplicated malaria (45.7%) and healthy controls (41.6%). Also, a case of severe malaria was almost twice as likely to be of type A as to be of type O (odds ratio (OR) 0.42, 95% confidence interval (CI) 0.20-0.88, P = 0.019), and more than twice as likely to be of type B as to be of type O (OR 0.38, 95% CI 0.16-0.89, P = 0.02). Furthermore, individuals with severe malaria were about six fold less likely to be of O as to be of type AB (OR 0.19, 95% CI 0.07-0.51, P = 0.0005). The study therefore revealed that patients with blood group O had a reduced chance of developing severe falciparum malaria as compared to patients with other blood groups (Tekeste and Petros, 2010).

2. Methodology

Study Design
This was a cross-sectional study that used purposive sampling to consecutively enroll qualified blood donors and typed their blood groups inorder to evaluate the association of ABO blood status and asymptomatic malaria infection among the donors.

Study area
The study was carried out in Asamankese Government Hospital in the malaria endemic region. Asamankese is a town in south Ghana and is the capital of West Akim Municipal District, a district in the Eastern Region of south Ghana. Asamankese has a 2013 settlement population of approximately 39,435. Asamankese is on the main highway to Kumasi and Accra in the interior.

With the coordinates: 5°52’N 0°49’W /5.867°N 0.817°W (Wikipedia)
Study Population
The study population composed of all qualified blood donors within the ages of 17 to 55 years of age who reported at the blood donation services of Asamankese hospital within November 2016 to April 2017.

Selection Criteria
- Exclusion criteria: Donors who had taken any antimalarial drugs within the last two weeks before the blood test. Donors who did not consent or assent to the study were also excluded.
- Inclusion criteria: All prescreened qualified donor and post screened unqualified blood donors who consent or assent to the study were also included.

Laboratory assessment, collection and diagnosis
Prior to bleeding process of the donor, explanation about the study was given and a written informed consent forms were given to the donors. Immediately after the bleeding is done small portion of the bled blood is collected into an ethylene diamine tetra acetic tube (EDTA), a thick and thin film with 6ul and 2ul of blood respectively was prepared on grease free labeled slide using a smooth edged slide spreader. Thin film was then fixed with methanol. The blood film was stained with 10% Giemsa that is 1 in 10 dilutions for 10 minutes. Finally, the films were examined under an oil immersion microscope objective (100x). Parasitaemia was determined for donors who tested positive for malaria by counting the number of parasites (asexual forms only) against 200 white blood cells (WBC). This counting was done by using hand tally counters. Then, the number of parasites per microliter of blood was calculated.

It is recommended in routine practice that parasite count be performed against 200 or 500 WBCs. If after counting 200 WBCs, 100 or more parasites are found, record the results in terms of number of parasites /200 WBC. If less than 100 parasites are found after counting 200 WBCs, parasite quantification should be continued until 500 WBCs are counted. This method of quantification is useful in low and moderate parasitaemia.
Figure 3: A, illustrate a prepared thick and thin blood film. Figure B, demonstrate a microscopic view of plasmodium Species in a thick blood film (Gatton and Group, 2015)

**Calculations of parasitaemia**

- Assume 8000 WBCs per microliter:
  - \( \frac{8000 \text{ WBCs/ul}}{\text{WBCs counted}} \times \frac{\text{parasites counted}}{\text{parasites counted}} \) WBCs counted

The blood group of the study participants was determined using the tile technique. Three spot of blood from each subject were made on a white plain tile and a drop of each antiserum A, B and D was applied to each spot respectively the mixture was further stirred with a plastic stirrer and rock for some time. Sign of agglutination were observed showing red pigment. Antisera D were used to determine the rhesus factor.

**Sample size determination**

\[
n = \frac{(N)}{[1+N(e)^2]}
\]

where

- \( n \) = the required sample size
- \( N \) = number of issue under study
- \( e \) = the margin of error will be 0.05

\[
n = \frac{(600)}{[1+600(0.05)^2]}
\]

\[
n = 240
\]

**Quality Control Measures**

1) Factors that might limit the generalizability of the results were considered, thus;

a) The specimens used in the study were collected from a population similar to the population in which the test will be conducted.

b) The credibility (performance characteristics) of the gold standards that were available to characterize the specimens was checked.

c) We checked whether the specimens have been stored appropriately.

2) The lot number and expiry date were checked.

3) Correct storage conditions of the test kits, as stated by the manufacturer, were ensured. The test kits were stored in a 2-8°C refrigerator.

4) From the refrigerator, in which the test kits and specimens were stored, they were brought to room temperature approximately 30 minutes before use. Test kits were opened only when they had reached room temperature, before use.

5) We ensure fresh romanowsky working solution were use.
Data Analysis plan

Data was entered into Microsoft Excel, and analyzed using SPSS version 22 and Graph Pad Prism. Results were presented in tables and figures.

Ethical Consideration

Ethical clearance for the study was obtained from the Committee on Human Research Publication and Ethics (CHRPE) of the School. Written informed consent was obtained from each study participant after the study objectives have been duly explained in the English language or a local dialect before recruitment.

3. Results

Table 4.1 below shows the socio-demographic characteristics of study participants. Most (48.6%) were in the age group, ≤20 years with least proportion (5.6%) ≥31 years. Majority (86.1%) were males with 92.3% married. More than half (59.4%) had had secondary school education. Unemployed and employed accounted for 59.4% and 27.2% respectively. Furthermore, only 4.3% used insecticide treated net.

Table 2 below shows blood donation characteristics of the study participants. Most (48.6%) had had at least one donation in their lifetime. Blood group ‘O’ were in the majority (58.2%) and rhesus positive people accounted for 91.0%. Moreover, paid donors were in the majority (45.8%) whereas voluntary and family replacement donors accounted for 37.5% and 16.7% respectively.

Table 2: Blood donation characteristics of study participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of blood donation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>3</td>
<td>0.9</td>
</tr>
<tr>
<td>Once</td>
<td>157</td>
<td>48.6</td>
</tr>
<tr>
<td>&gt;2 - 4 times</td>
<td>140</td>
<td>43.3</td>
</tr>
<tr>
<td>&gt;4 times</td>
<td>23</td>
<td>7.1</td>
</tr>
<tr>
<td>ABO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>78</td>
<td>24.1</td>
</tr>
<tr>
<td>B</td>
<td>53</td>
<td>16.4</td>
</tr>
<tr>
<td>AB</td>
<td>4</td>
<td>1.2</td>
</tr>
<tr>
<td>O</td>
<td>188</td>
<td>58.2</td>
</tr>
<tr>
<td>Rhesus</td>
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<td></td>
</tr>
<tr>
<td>Negative</td>
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<td>9.0</td>
</tr>
<tr>
<td>Positive</td>
<td>294</td>
<td>91.0</td>
</tr>
<tr>
<td>Donor type</td>
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<tr>
<td>Voluntary</td>
<td>121</td>
<td>37.5</td>
</tr>
<tr>
<td>Paid</td>
<td>148</td>
<td>45.8</td>
</tr>
<tr>
<td>family replacement</td>
<td>54</td>
<td>16.7</td>
</tr>
</tbody>
</table>

Prevalence of malaria infection among the studied donors was 2.8%.

Figure 5: Prevalence of malaria infection among blood donors at the Asamankese Government Hospital

Figure 6: Malaria parasitaemia comparison among ABO blood groups of study participants

Prevalence of malaria infection among the studied donors was 2.8%.
From figure 4.2 above, malaria density in AB blood group was higher compared to the other ABO blood groups. However, no significant difference was found in malaria density across the blood groups (p = 0.4324).

![Figure 7: Malaria density comparison between rhesus positive and negative blood donors](image)

From fig 4.3 above, though statistically insignificant, rhesus positive donors had higher malaria density compared to the rhesus negatives (p = 0.333).

![Table 3: Blood group of donors stratified by gender](image)

From table 4.3 above, most of the blood groups O were males (60.0%) and females (46.7%). There was however no significant different in blood groups between males and females (p = 0.284). Rhesus blood group type was significantly associated with gender (p = 0.038).

![Figure 8: Regression graph of age and parasite density of study participants](image)

From figure 4.2 above, malaria density in AB blood group was higher compared to the other ABO blood groups. However, no significant difference was found in malaria density across the blood groups (p = 0.4324).

Among the age groups, ≤20years had the highest malaria infection rate (3.8%). Though statistically insignificant, age groups 21 – 25 years and 26 – 30 years were associated with decreasing odds of malaria infection [(OR = 0.5, p = 0.386) and (OR = 0.6, p = 0.640) respectively]. Moreover, females were associated with increasing odds of malaria infection compared to males (OR = 3.2, p = 0.107). Compared to ‘O’ blood groups, blood group AB is significantly associated with increasing odds of malaria infection (OR = 15, p = 0.030). Furthermore, rhesus positive blood group donors were significantly associated with decreasing odds of malaria infection (OR = 0.2, p = 0.020).

![Table 4: Factors associated with malaria infection of blood donors](image)

*RMI: rate of malaria infection, OR: odds ratio, CI; confidence interval, ITN; insecticide treated net usage

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From fig 8 above, age had a positive correlation with malaria density (p = 0.433). Age account for about only 9% in the variation of malaria density (R-square = 0.09).

4. Discussion

Asymptomatic malaria remains a challenge for malaria control programs as it significantly influences transmission dynamics. Determination of the various ABO/Rh blood group distributions and their association with malaria infection has paramount importance in the context of transfusion medicine and malaria control. This study therefore sought to find out the prevalence of Plasmodium falciparum infection and their association with ABO/Rh blood group among donors of Asamankese General Hospital.

Most of the study donors recruited were males and were in the age group ≤20 years (Table 4.1). Prevalence of plasmodium falciparum malaria infection was 2.8% (Fig 4.1). This is comparatively lower compared to a facility based cross-sectional study conducted in Ethiopia which recorded a prevalence of 4.1%, and in Ghana in which 4.7% was recorded (Alemu et al., 2013). In sharp contrast is another cross-sectional study from Nigeria in which 36.4% prevalence was reported (Abah et al., 2016). The different geographical locations of all these could account for the variations in the proportions. When analyzed from the point that recipients are already compromised and weakened with existing ailments, the seemingly low malaria prevalence is alarming. Recipients of this blood are also those that are vulnerable; mostly pregnant women, children under 5 years, accident victims and other immuno-suppressive patients. Moreover, inaccuracies from microscopic diagnoses looking at its subjectivity and inherent low sensitivity could misdiagnose most of the asymptomatic malaria carriers. This is evidenced by findings from Ghana that a prevalence of 4.7% by microscopy increased to 18% when diagnosed using polymerase chain reaction (Onabanjo et al., 2012; Alemu et al., 2013).

Proportion of blood groups A, B, AB, and O were 24.1%, 16.4%, 1.2% and 58.2% respectively. Rhesus positive donors on the other hand accounted for 91.0% (Table 4.2). This shows that blood group O is the most prevalent in this part of the world and is in keeping with findings by (Otajevwo, 1997; Onabanjo et al., 2012; Abah et al., 2016) all from Nigeria. Similarly, (Oladeinde et al., 2014) reported that because of endemicity of malaria in Africa, more than half of the population belongs to blood group O which protects against malaria (Benedict et al., 2012).

It has long been known that people with blood type O are protected from dying of severe malaria and that blood group O provides protection against severe malaria (Abah et al., 2016). This may explain why blood type O seems to be the commonest blood type in malaria. In this study, malaria infection rate was (2.1%) among the blood group O participants compared to the other groups which recorded 3.8% for blood group A, 1.9% for blood group B and 25% for blood group AB. In addition, blood groups A and AB were associated with increased odds of malaria infection (OR = 1.8, 15.0 and P = 0.432, 0.030). Hence findings in the present study substantiate the hypothesis that P. falciparum has evolutionarily shaped the distribution of ABO phenotype. However, other studies in malaria endemic regions like India show that B phenotype is the most abundant ABO blood group suggesting that the above scenario is not exclusive (Benedict et al., 2012).

Age of study participants correlated positively and accounted for about 9.0% in the variations in malaria density (R-square = 0.09) though statistically insignificant (p = 0.433) (fig 4.3). However malaria parasite density was significantly affected by age and blood donors within the age group of 21-26 years were observed to have the highest parasite count in a cross-sectional study conducted in Nigeria (Oladeinde et al., 2014). This is also in line with a previous report (wikipedia). The WHO reports that young people in malaria endemic areas of sub-Saharan Africa are more disposed to malaria infection than older individuals (Organization, 2008).

The WHO recommends the recruitment of volunteer non-renumerated blood donors from low-risk populations to ensure the safety of transfused blood (Kasraian and Maghsudlu, 2012). Several studies have reported that infectious diseases are more prevalent among donors who are recruited by monetary incentives (28-30) as they may intentionally fail to identify high risk behaviors during the donation interview in order to obtain the incentive offered (Kasraian and Maghsudlu, 2012; Tadesse and Tadesse, 2013). In this study however, paid donors were in the majority (45.8%) (Table 4.2). Paid donors were associated with increased odds malaria rate though statistically insignificant (OR = 1.5, p = 0.322). This is comparable with a study in which commercial blood donors were observed to have a higher prevalence of asymptomatic malaria infection than voluntary blood donors (Oladeinde et al., 2014).

Most of the study participants were Rhesus positive (91.0%) in this study which goes in line with previous studies (Onabanjo et al., 2012; Tadesse and Tadesse, 2013). Rhesus positive blood group was significantly associated with decreased risk of malaria infection (OR = 0.2, p = 0.020) (Table 4.4). This confirms the hypothesis that the rhesus genotype D is protective against malaria infection and therefore evolutionarily affects blood group distribution. However, previous studies found no association of rhesus blood group with asymptomatic malaria infection. Though this study has found considerable results, a large sample size could have thrown more light on the prevalence of asymptomatic malaria and their association with ABO/Rh blood groups among the studied donors.

5. Conclusion

Considering the vulnerable nature of blood recipients, there is a high prevalence of asymptomatic malaria among blood donors of Asamankese Government Hospital. The highest proportion of the donors is in the blood group O and with Rh positive. Moreover, donors with blood groups O and Rh D appear to be significantly protected from asymptomatic malaria infection. There is also overwhelmingly high percentage of paid blood donors at the Asamankese government hospital.
6. Recommendation

I recommend a more sensitive diagnostic tool in screening blood donors at the Asamankese Government Hospital to offset the transmission of malaria from donors to recipients. In addition, a large scale study involving large sample size is warranted to buttress the findings from this study.

Conflict of Interest: Non Declared

Source of Funding: Self-Funded

References


[31] wikipedia Renin–angiotensin system.