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Comparative Analysis of Malaria Diagnostic Tests and Prevalence of Malaria Infection among Blood Donors at General Hospital, Hadejia, Jigawa State, Nigeria

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Abstract
Blood for transfusion is not routinely tested for malaria in Nigeria despite the recommendation by World Health Organization (WHO) that all donated blood should be tested for malaria where appropriate and possible and that there should be quality assured testing for transfusion-transmissible infection. A hospital based cross sectional descriptive study was carried out to screen for malaria parasites using microscopy and rapid diagnostic tests among consented blood donors at General Hospital, Hadejia, Jigawa State, Nigeria. Four hundred, 400 blood samples were collected from apparently healthy blood donors (398 Males, 2 Females) who presented with no overt signs and symptoms of malaria and routinely screened free of Hepatitis B Virus, Hepatitis C Virus, Syphilis, HIV I and II Virus. The samples were screened for malaria parasites using CareStartTm Malaria HRP2 One Step Rapid Diagnostic Test (RDT) and Microscopy, and blood group determined using ABO blood grouping system. Out of the 400 samples screened 107 (26.7%) were found to be positive using Microscopy with a density of +++ trophozoite in 1 (0.2%) blood donor, ++ in 2 (0.5%) blood donors, and + in 104 (26%) blood donors with an overall prevalence of 26.7%. Fourteen (3.5%) blood donors were positive by RDT with 3 false positive RDT. High positivity rate was found among blood donors with blood group O+ (48.1%), majority were farmers and from the age group 18 – 25 years with the highest prevalent rate of 46.2% and 39.3% respectively. The most preventive measures taken were the use of insecticide treated bed-nets. Most of the blood donors (49.8%) have primary level of education (western). Blood film was found to be highly sensitive than RDT with a positivity rate of 26.7% and 3.5% respectively. These were however statistically significantly different (P<0.05).
Key Words: Malaria, Parasites, Blood Transfusion, Microscopy, Rapid Diagnostic Test

INTRODUCTION

In Nigeria, blood for transfusion is not tested for malaria despite the recommendation by World Health Organization (WHO) that all donated blood should be tested for malaria where appropriate and possible and that there should be quality assured testing for transfusion-transmissible infection. 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 Nigeria (Federal Ministry of Health Nigeria, 1991; Attah, 2000). Malaria by blood transfusion route is a potential health hazard but is often neglected in many malaria endemic areas. The diagnosis of malaria in blood transfused patients is unexpected and this is often missed. Malaria transmitted by blood donation is a problem because a small number of parasites transmitted by blood transfusion can cause malaria infection as the blood transfused patient is already weak by severe disease (Federal Ministry of Health Nigeria, 1991).

Malaria transmission by blood transfusion could poses a threat in Nigeria which is among the malaria endemic countries as the dominant parasite in the country is the deadliest (*Plasmodium falciparum*) which can lead to fatality. Whole blood is used for transfusion in Nigeria which is one of the most common sources of malaria by blood transfusion (Owusu *et al.*, 2010). The soil in most part of Hadejia town is a clay soil which due to its water retaining ability provides breeding sites for mosquitoes. The town and neighbouring villages are mainly farmers and they engage in whole year farming, irrigational farming is very common in this part because of the presence of water bodies.

These prevailing environmental condition aids in building a suitable environment for mosquito breeding there by increasing the rate of malaria transmission.

As the blood donors are healthy individuals, the malaria density is very low but can be fatal to transfused patient as they are already weakened by other underlying diseases. According to CDC (2012), Nigeria is highly endemic for malaria parasite with highest reported cases of death in the whole world and still malaria screening of blood donors is not done even though WHO recommended that all blood for donation should be screened for malaria parasite where appropriate and possible (WHO, 2009).

All malarial parasites species can survive in stored blood for at least 1 week and even longer in frozen blood (Lakshmi & Anuradha, 2015), this makes malaria transmitted through blood transmission a serious case as only a small number of infected red cells from donor can lead to malaria in the recipient and the diagnosis is often missed and unexpected in a patient who is otherwise critically sick. Malaria transmitted by blood transfusion is also common particularly common in countries where blood donor come from less affluent class (Lakshmi & Anuradha, 2015). In order to have a sustained control, malaria diagnostic methods needs to be sensitive and specific, easy and rapid (Beyene *et al.*, 2012).

The studied area is known to be malaria endemic due to its agricultural activities, water bodies, and clay nature of its soil that makes it not to easily absorb water leading to many potholes in the area which are good mosquito breeding sites. This brings the need for the screening of donors for malaria prior to donation. Since healthy donors are selected for blood donation, density of parasite is usually less if present and hence may be missed. A sensitive method for the detection of malaria parasite needs to be introduced in each blood bank to prevent malaria by blood transfusion. This procedure will reduce the risk of induced malaria by blood donors and will not impede donation flow. A sensitive detection method can be easily introduced in each blood bank for malaria screening. Screening of blood donors for malaria with a sensitive diagnostic method will minimize the risk of transmission of malaria by blood transfusion. Hence the need for the study that was aimed to determine the prevalence of malaria by blood transfusion and sensitivity of malaria screening methods.

MATERIALS AND METHODS

Study Area

The study was conducted at Hadejia General Hospital, Hadejia, Jigawa State. It is one of the largest hospitals in the state where patient comes from neighboring villages. The people are mainly farmers, fishermen and herdsmen. The area is known to be malaria endemic due to the large number of Anopheles mosquitoes found. Hadejia is located between 12^{o} 27 north of the equator and between 10^{o} 2 east of the Greenwich meridian. It falls into Sudan-Sahelian vegetation zone and has a surface temperature of 25-30^{o}C with lower night temperatures especially during harmattan. It has a low relative humidity of about 20-40% (Encyclopædia, 2006). The area has malaria infection occurrence throughout the year with high rate (of malaria) from August to October due to heavy rainfall season this reduce from November to February due to the cold season. It has an all season breeding of Anopheles mosquitoes (Encyclopædia, 2006).

.Ethical Consideration.

An ethical clearance was obtained from the management of Hadejia General Hospital, Jigawa State to conduct the study.

Informed Consent

An informed consent form was given to the blood donors indicating the donor's name, signature and date. The purpose, risk, method and benefit of the research were fully explained to the blood donors and their informed consent was taken.

Sample Size

The sample size was determined by an epidemiological formula put forward by Lwanga and Lemeshow, 1991 using local prevalence based study done in Abakalilki, Nigeria by Epid *et al.,* 2008.

Sample size= $n=\frac{Z^2PQ}{L^2}$

n= minimum sample

Z= standard distribution at 95% confidence interval= 1.96

P= study on past review 51.1% (Epid *et al.*, 2008)

Q =1-p

L= Allowable error, taken as 0.05

Substituting the formula we have $n=\frac{1.96^2 \times 0.5 \times 1-(0.515)}{0.05^2}$

n = 373

373 + 5% attrition= $\frac{373 \times 5}{100} = 392$

The sample size determined was 392. However, the size was rounded to the nearest hundred. Therefore, 400 blood donors were used for the study.

Study Design

The study was a hospital based cross sectional descriptive study conducted between February to August, 2016.

Study Population

The study subjects were apparently healthy blood donors and close relative to the patients that need blood for transfusion, presented to the hospital with no overt signs and symptoms of malaria, and found to be free after routinely screened of Hepatitis B Virus, Hepatitis C Virus, Syphilis HIV I and II Virus. Majority of the blood donors were from the neighbouring villages with few of them from within Hadejia town. Three hundred and ninety eight (398) of the blood donors were males with only 2 females which can be due to the fact that females hardly donate blood in this part of the country.

Sample Collection

Venous blood was collected using an ethylenediamine tetra acetic acid (EDTA) container. Numbers from 001-400 were used to indicate the blood samples in order to maintain the patients anonymity.

Sample Analysis

Smear Making

Thick and thin blood films were made on the same slide. Thin films were made by putting a drop of blood on a glass slide and using another clean slide as the spreader, the slide was held at 45° angle, toward the drop of blood on the specimen slide until the blood spreads all on the entire width of the spreader slide. While holding the spreader slide at the same angle, it was pushed forward rapidly and smoothly. Above the thin film, Thick film was made by using the corner of a clean slide as a spreader to spread the drop of blood in a circular motion. The film was allowed to air dried. The slides were then labeled using appropriate numbers. After drying, the thin films were fixed in absolute methanol by dipping the slide carefully in a vertical position and without the thick films touching the methanol. They were then air dried with the thick film up (Cheesebrough, 2004).

Giemsa Staining

Two (2) ml of commercial stock giemsa solution was added to 40ml of buffered water (pH7.2) in a coplin jar. The entire slides were stained with diluted giemsa stain in a coplin jar. The slides were placed in the coplin jar with the thick film down for 45minutes. The films were washed with tap water separately and air dried in a vertical position (Cheesebrough, 2004).

Microscopy

The dried films were examined microscopically using ×100 oil immersion objective lens. The parasite density was obtained by counting the parasite. The parasite was counted using semi quantitative count scale (Cheesebrough, 2004).

Rapid Diagnostic Test (RDT)

These tests were done using CareStartTm Malaria HRP2 One Step Rapid Test which is a two-band RDT detecting Histidine Rich Protein 2 (HRP-2). Five (5) micro litre of the blood sample was added into the sample well and 3 drops of assay buffer were added into assay buffer wells. The blood -buffer mixture were allowed to run toward the test and control window. Result was read within 20 minutes. The present of two color bands indicated positive result. The present of one band only on the control line within the result window indicated negative result.

ABO Blood Grouping.

Three drops of the sample blood were placed separately on a grease free clean white tile on which a drop of antisera A, B and D was applied respectively (Murex. Inc. Dartford. UK). The blood cells and the antigen were mixed with applicator stick. The tile was then tilted to detect for agglutination.

Data Analysis.

Data obtained were analyzed using SPSS Software version 16 (SPSS INC. Chicago, IL, USA). Values were considered significant when the *P*- Value is less than 0.05 with respective confidence interval of 95%.

RESULTS

The results obtained from the study were presented in Tabular form, percentages and analyzed using Chi-square by SPSS software. Four hundred 400 samples were screened for malaria parasites. The number was made up of 398 (99.5) males and 2 females (0.5) blood donors that were found to be free after routinely screened of Hepatitis B Virus, Hepatitis C Virus, Syphilis, HIV I and II Virus. A microscopy density of +++ trophozoite was found in 1 (0.2%) blood donor, ++ in 2 (0.5%) blood donors, and + in 104 (26%) blood donors. No parasite was found in 293 blood donors.

Fourteen (14) blood donors were found positive for malaria parasite by RDT as shown in Table 1. In relation to blood group, malaria parasite was found in 48.7%, 16.8% , 30.8%, 3.7%, blood donors of blood group O+, A+, B+, AB+ respectively. No parasite was found in the only blood donor that was found to be having a blood group of AB-. Only blood group O+ blood donors have parasite density of ++ in 2 of the blood donors and +++ in 1 of the blood donor. All the other positive blood donors have malaria parasite density of + microscopically as shown in Table 2.

Out of the 400 blood donors, 312 (78%) were from villages within the emirates and 88 (22%) were from Hadejia town.

Three (3) blood donors were found to be RDT positive but no parasite was found microscopically (false positive) as shown in table 6. Most of the blood donors 199 (49.8%) have primary level education, 111 (27.7%) have secondary level with 28 (7%) blood donors reaching up to tertiary level. However, 62 (15.5%) of the blood donors have no western education as shown in Table 3.

Table 4 shows malaria parasite distribution based on occupation by Blood film and was found to be 11.2%, 10.3%, 6.5%, 44.9%, 4.7%, 9.3%, 7.5%, 5.6%, among businessmen, craftsmen, commercial drivers, farmers, civil servant, fishermen, herdsmen and others respectively with a density of ++ found in one farmer, one other and +++ found in one herdsman.

The number of blood donors examined using Blood film for an age group of 18-25 was 172 (43.2%), 42 positive, 112 (28%) for 26-30 with 36 positive, 55 (13.8%) for 31-35 with 12 positive, 32 (7.7%) for 36-40 with 8 positive, 24 (6%) for 41-45 with 8 positive and 5 (1.3%) for 41-50 with no parasite found (Table 5).

The most preventive measures taken was the use of insecticide treated bed-nets (46%), followed by the use of insecticide (19%), traditional methods (15%), door and window nets (14%) and use of mosquito repellant (8%). None of the donors uses prophylaxis as a preventive measure. RDT and Blood film have a positivity rate of 3.5% and 26.7% respectively. The Chi square test does not conform to the null hypothesis that there is no significant difference in sensitivity and specificity between Blood film and Rapid diagnostic test ($P \geq 0.04$).

Table 1: Malaria Infection in Relation to RDT and Blood Film

RDT	Blood Film			Total	
	+ (%)	++ (%)	+++(%)	NPF (%)	
Negative	94 (24.6)	1 (0.0%)	1 (0.3)	290 (75.1)	386
Positive	10 (71.4)	1 (0.0)	0 (7.1)	3 (21.4)	14
Total	**104**	**2**	**1**	**293**	**400**

Key:
+ When there are 1-10 asexual parasites per 100 film field.
++ When there are 11-100 asexual parasites per 100 film field.
+++ When there are 1-10 asexual parasites per single film field.

Table 2: Distribution of Malaria Parasite by Blood Group among Blood Donors by Blood Film

	Blood Film					
Blood Group	**+**	**++**	**+++**	**Percentage**	**NPF**	**Total**
O+	49	2	1	48.7	139	191
A+	18	0	0	16.8	64	82
B+	33	0	0	30.8	77	109
AB+	4	0	0	3.7	13	17
AB-	0	0	0	1	1	
Percentage	26.0	0.5	0.2	73.3	100	
Total	104	2	1	100	294	400

Key:
+ When there are 1-10 asexual parasites per 100 film field.
++ When there are 11-100 asexual parasites per 100 film field.
+++ When there are 1-10 asexual parasites per single film field

Table 3: Educational Level among Blood Donors

Western Educational Level	Blood Donors	Percentage
Primary	199	49.8
Secondary	111	27.7
Tertiary	28	7
No Formal Education	62	15.5
Total	400	100

Table 4: Malaria Parasite Distribution Based on Occupation (Blood Film)

Occupation	Microscopy				Total	Percentage (Positive)
	+	++	+++	NPF		
Businessmen	12	0	0	29	41	11.2%
Craftsmen	11	0	0	22	33	10.3%
Commercial drivers	7	0	0	27	34	6.5%
Farmers	48	0	0	133	11	44.9%
Civil servant	4	1	0	13	18	4.7%
Fishermen	10	0	0	38	48	9.3%
Herdsmen	7	0	1	17	25	7.5%
Other	5	1	0	14	20	5.6%
Total	104	2	1	293	400	100%

Key:
+ When there are 1-10 asexual parasites per 100 film field.
++ When there are 11-100 asexual parasites per 100 film field.
+++ When there are 1-10 asexual parasites per single film field

Table 5: Malaria Parasite Distribution Based on Age Group (Blood Film)

Age Group	+	++	+++	NPF	% Positive	Total	%Total
18-25	40	2	0	131	39.3	173	43.2
26-30	36	0	0	76	33.6	112	28
31-35	12	0	1	42	12.1	55	13.8
36-40	8	0	0	23	7.5	31	7.7
41-45	8	0	0	16	7.5	24	6
46-50	0	0	0	5	0	5	1.3
Percentage	26.0%	0.5%	0.2%	73.2%	100		
Total	104	2	1	293	100	400	100

Key:
+ When there are 1-10 asexual parasites per 100 film field.
++ When there are 11-100 asexual parasites per 100 film field.
+++ When there are 1-10 asexual parasites per single film field

Table 6: Malaria Positive RDTs seen Negative by Blood Film (False Negative)

Lab Number	RDT	Blood Film
011	Positive	NPF
021	Positive	NPF
098	Positive	NPF
Total	3	3

DISCUSSION

Out of the total of 400 blood samples examined, 107 (26.7%) were found to be positive by Blood film which is lower than the positivity rate found in a study by Ekwunife *et al.*, (2011) in Onitsha which shows a high positivity rate of 74.1%. Epid *et al.*, (2008) also shows a higher positivity rate of 51.5% in his study in Abakaliki. An almost similar positivity rate (28%) was found in a study by Abah and Joe (2016) in Portharcourt and Agboola *et al.*, (2010) in Lagos. A positivity rate of 25.9% was found in a study conducted in Benin City by Bankole *et al.*, (2014) which is nearly similar to the rate found in this study. Another higher malaria infection rate of 40.9% was found in a study done in southeastern part of Nigeria by Uneka *et al.*, (2006). Majority of the blood donors in this study are age group 18-25 years which has the highest positivity rate followed by 26-30 years and this shows some similarity with a study by Lakhshmi and Anuradha, (2015) in India having the highest donor population in age group of 23-27 years, a study by Uneka *et al.*,, (2006) in south eastern part of Nigeria also shows that individuals aged 20–25 years were most infected. Similar rate was found in a study by Bankole *et al.*, (2014) among age group 21-26 years and Abah and Joe (2016) found theirs among 21-30 years. These categorize most blood donors as youth.

A study by Abioye *et al.*, (2014) in Abuja shows a highest prevalent rate in blood donors of 31-40 years age group which is in contrary to what was found in this study. Based on occupation,

Farmers have the highest infection rate of 45.2%, which can be due to the fact that they live close to their farms. Malaria parasite was found positive at a percentage rate of 3.5% and 26.7% by Care Start Tm Malaria HRP2 test and Blood film respectively. This is less in comparison with a study by Sheyin and Bigwan, (2013) that compare the diagnostic performance of care start malaria rapid diagnostic test (RDT) with reference microscopy in patients which shows a rate of 52.9% by Blood film and 42.6% by Care Start Tm Malaria HRP2 Test. Also a higher rate of 40.9% for Blood film and 39.4% for CareStartTM RDT was reported by Bayene *et al.,* (2012).

In this study, blood groups O+ were most infected followed by B+, A+ and AB+. This is similar to what was obtained in a study by Abah and Joe in (2016) which also shows high prevalence among O+ donors (60.7%) and AB been the lowest (5.4%). Bankole *et al.,* (2014), Agboola *et al.,* (2010), Ekwunife *et al.,* (2011), Epid *et al.,* (2008) also found malaria to be more prevalent among blood group O+ donors. Blood film shows high sensitivity for detection of malaria parasites than the RDT in both studies but in this study, RDT positivity was very low compared to both studies as this study involve healthy blood donors with a very low parasite density.

This study shows a positivity rate of 26.5% using Blood film and 3.5% by RDT which is lower than what was found in a study by Kennedy and Ibinabo, (2015) which shows a positivity rate of 67.5% by Blood film and 15% by RDT. A similar study by Sheyin and Bigwan (2013) in Zaria shows a positivity rate of 52.9% by Blood film and 42.6% by CARE START HRP2 RDT. Shelin and Bigwan, (2013) also shows a false negative of 30 sample using Blood film in their study while comparing CARE START HRP2 rapid malaria test with light microscopy for guiding patient's treatment of fever. In this study 3 blood donors were found positive using RDT and negative by Blood film which is less than the false negative reported by Shelin and Bigwan, 2013.

CONCLUSION

Malaria parasite was found to be prevalent among the blood donors. Majority of the blood donors are from the neighbouring villages within the emirate. The CareStartTM HRP2 Rapid Diagnostic Test (RDT) kit has a very low sensitivity as compared to Blood film and also since a false positive RDT was detected, it cannot be used as a substitute for microscopy. Youths are more exposed to malaria infection than others as they are more exposed to mosquito bites probably due to their habit of hanging out outdoors at night or due to their occupation. Blood group O+ has the highest infection rate. Farmers were found to be more exposed to malaria infection in this study and the most preventive measures taken is the use of insecticide treated bed nets.

RECOMMENDATIONS

As blood donors are healthy individuals with no clinical symptoms of malaria and has low parasite density, a cheap and sensitive method that can detect a very low parasite density should be used for daily screening of malaria in blood banks as the blood transfusion recipient are already weakened by other disease and can easily developed malaria infection if transfused with malaria infected blood units. Each and every blood donor should be screened for malaria parasite prior to donation. Blood film method of malaria detection should be introduced to the blood bank because of its high sensitivity as compared to RDT. An invitro method of killing the malaria parasite in blood units needs to be employed as the high demand of blood for donation and its shortage necessitates the infected blood unit not to be rejected. Infected blood donors also need to be treated and transfused patient should be given curative antimalarial drugs followed by prophylactic drugs.

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