Scholars Journal of Applied Medical Sciences (SJAMS)
Abbreviated Key Title: Sch. J. App. Med. Sci.
ISSN 2320-6691 (Online)
ISSN 2347-954X (Print)

A Unit of Scholars Academic and Scientific Society, India
www.saspublishers.com

Medical Laboratory

Prevalence of Malaria Parasites among Patients Attending Some Selected Health Institutions in Kaduna State, Nigeria

Akogwu S[1*], Uhunmwangho EJ[2], Garba DD[1], Emelike OF[2], Amaechi R[2]
[1]School of Medical Laboratory Science, Ahmadu Bello University Teaching Hospital (ABUTH), Shika- Zaria, Nigeria
[2]Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, Ambrose Alli University, Ekpoma, Nigeria

Original Research Article

***Corresponding author**
Akogwu S

Article History
Received: 11.11.2018
Accepted: 23.11.2018
Published: 30.11.2018

DOI:
10.36347/sjams.2018.v06i11.073

Abstract: This study has been designed and under taken to determine the prevalence of malaria parasites among patients attending some selected Health Institutions in Kaduna state, Nigeria and assess the diagnostic performance of a rapid diagnostic test (RDT). A total 300 patients whose ages ranged from < 10 - > 60 years were investigated. Two point five milli litres of venous blood were collected from the ante-cubical vein into 0.04ml ethylene diamine tetra acetic acid (EDTA) bottle. Laboratory analysis was conducted under standardized conditions using microscopy and Rapid diagnostic Test (RDT). Out of the 300 patients examined, 76 (25.3%) were parasitaemic by microscopy with an average parasite density of 240/µl of blood. The rapid diagnostic tests (RDTs) detected antigenaemia in 81 (27%) patients. The prevalence of the infection was not significantly associated with some of the socio-economic and demographic factors considered. On the other hand, 70 (23.3%) patients were positive by both RDT and microscopy (True positives) while 213 (71%) were negative by both methods (True negatives). The result also showed that 11 were negative by microscopy but positive for RDT (False positives) whereas 6 were negative by RDT but positive by microscopy (False negatives). There was statistically significant agreement between the two diagnostic methods ($k = 0.853$; $p < 0.001$). In this study, we demonstrate that RDT (SD BIO LINE) kit is reliable, rapid, easy to use and simple to interpret. The RDT is a valuable tool to complement microscopy in places where experienced laboratorians are lacking and facilities for microscopy are poor. In line with this, we concur to earlier recommendations for the use of the RDT kit in the management of febrile patients in malaria endemic regions and for epidemiological studies.
Keywords: Prevalence, malaria, patient, parasitaemia, microscopy, antigenaemia.

INTRODUCTION

Malaria is a life-threatening, blood disease caused by *Plasmodium* parasites that are transmitted to people mainly through the bites of infected female *Anopheles* mosquitoes. There are 5 parasite species that cause malaria in human namely- *P. falciparum*, *P. malariae*, *P. vivax*, *P. ovale* and *P. knowlesi.* Only two out of these species – *P. falciparum* and *P. vivax* – pose the greatest threat [1]. *P. falciparum* is the most prevalent malaria parasite on the African continent. It is responsible for most malaria-related deaths globally. *P. vivax* is the dominant malaria parasite in most countries outside of sub-Saharan Africa [1].

Other comparatively rare mechanisms for transmission include: congenitally-acquired disease, blood transfusion, sharing of contaminated needles, organ transplantation, and nosocomial transmission [2-4].

The recent report by Center for Disease Control [5] stated that 3.2 billion people live in areas at risk of malaria transmission in 91 countries and territories. The World Health Organization, estimates that in 2016 malaria caused 216 million clinical episodes, and 445,000 deaths. Malaria is a major public health problem in Nigeria where it accounts for more cases and deaths than any other country in the world [6].

Malaria is a risk for 97% of Nigeria's population. There are an estimated 100 million malaria cases with over 300,000 deaths per year in Nigeria [7]. Malaria contributes to an estimated 11% of maternal mortality, accounts for 60% of outpatient visits and 30% of hospitalizations among children under five years of age in Nigeria.

Since 2010, the World Health Organization (WHO) has recommended that all suspected malaria cases receive a parasitological diagnosis, either by microscopy or a rapid diagnostic test (RDT), prior to treatment with artemisinin-based combination therapy (ACT). Rapid and effective malaria diagnosis not only alleviates suffering, but also decreases community transmission. The non-specific nature of clinical signs and symptom of malaria may result in over-treatment of malaria or non-treatment of other diseases in malaria endemic areas [8].

Light microscopy is the traditional reference standard for the diagnosis of malaria [9]. The sensitivity of this method can be excellent, with detection of malaria parasite densities as low as 5 to 10 parasites/µl of blood (approximately 0.0001% parasitaemia) [10]. Microscopy permits determination of the infecting species as well as the stage of the circulating parasites. In addition, circulating parasite density can be determined, which may aid in prognosis, and serial examinations can monitor the parasitological response to chemotherapy. Examining an individual sample is relatively inexpensive, but this cost may not incorporate the health care system's cost of equipment and training. Finally, malaria smears provide a permanent record for quality assessment of the microscopy diagnosis. However, the vast majority of malaria episodes occur in rural areas of sub-Saharan Africa, where microscopy is often unavailable and shortages of trained personnel further limit its use as a valid diagnostic method [11, 12].

Microscopy also possesses certain limitations such as requirement of well-trained microscopist usually not available in most of the rural centers and remote areas and also takes time, which causes delay in the treatment of malarial cases. It needs careful preparation and application of reagents to ensure quality results etc. These diagnostic limitations affect the medical care provided, as malaria is a potentially fatal disease, usually curable if diagnosed quickly [10, 13]. The urgency and importance of obtaining results quickly from the examination of blood samples from patients with suspected acute malaria is now made possible with the introduction of rapid malaria diagnostic tests (RDTs) [13].

Immuno- chromatographic RDTs are low-cost, simple tools for the diagnosis of malaria. In contrast to microscopy, RDTs require minimal infrastructure, can be used by non-professional healthcare staff, and provide a timely result [14]. However, Immuno- chromatographic RDTs also possesses certain limitations such as higher positivity rate attributable to cross-reactivity with heterophile antibodies [15] and persistence of residual antigens for weeks after treatment [16, 17]. The false negative RDT may be due to low parasitaemia as in the majority of cases or by interpreting the RDT before the test line has fully developed [16]. The storage temperature the RDT product subjected to by marketers could cause low sensitivity. Exposure of RDT kit to high temperature, denaturation of antibodies in the test membrane can impair binding to the target antigen at high temperature, damage to the nitrocellulose membrane forming the strip thus changing its flow characteristics or causing the antibody to detach from the membrane and variation in the histidine rich-protien 2 (HRP 2) genotype of *P. falciparum* have been implicated as a possible cause of poor performance in the tropics [18, 19].

The overall aim of this research is to determine the prevalence of malaria parasites among patients attending some selected health institutions in Kaduna state and assess the diagnostic performance of SD BIO LINE RDTs.

MATERIALS AND METHODS

Study area

The study was carried out in Kaduna in north-western Nigeria. It is located at latitude 10^{o} 3’ 20’’N and longitude 7^{o} 26’ 17’’ E. Kaduna is bordered by the states of Zamfara, Katsina, and Kanoto the north; Bauchi and Plateau to the east; Nassarawa to the south; and Niger to the west. Abuja Federal Capital Territory also borders Kaduna state to the southwest. The three study facilities are situated in Kaduna South and Chikun local government areas respectively.

The Kaduna River, a tributary of the Niger River, flows roughly east to west through the centre of the state. The state’s natural vegetation consists largely of savanna woodlands. Almost all of the state’s Hausa and Fulani inhabitants are Muslims; in the south, however, there are about 30 other ethnic groups in the state, not all Muslim, of which the largest is the Gbari (Gwari). These areas have poor drainage due to indiscriminate refuse dump which makes for stagnant water thus providing good resting and breeding sites for malaria parasites mosquito vectors. Other factor that makes malarial endemicity high here is the poor socioeconomic status of the dwellers [20].

Research design

The research was a cross-sectional, facility-based study to determine the prevalence of malaria parasites among patients attending some selected health institutions in Kaduna state and assess the diagnostic performance of SD BIO LINE RDTs.

Study population

One hundred (100) samples each was collected from General Hospital Sabon-Tasha, Yusuf Dan Tsoho General Hospital, Tudun-wada and Gwamna Awan Genaral Hospital, Kakuri respectively. These hospitals render secondary care services for the Kaduna populace and its neighbouring towns.

Sample size

Sample size was determined using the method [21]

$n= Z^2 X P (1 – P) /D^2$

Where n = Sample size

P = expected prevalence = 84% [22].

D = Precision at 5% (0.05)

Thus $n = (1.96)^2 x 0.847 x (1- 0.153) / (0.05)^2$

$= 3.8416 x 0.847 x 0.153 / 0.0025$

$= 0.4978 / 0.0025$

$= 199.13$

Hence, n= 199.13 Attrition rate of 51% = 101.49. Therefore, sample size = 199.13 + 101.49 = 300.

Subject selection criteria

Incusion criteria

All patients who presented febrile condition suggestive of malaria parasites that after seeing doctor were sent to the laboratory for investigation were recruited.

Exclusion criteria

All patients who did not present febrile conditions suggestive of malaria parasites and who were not sent to the laboratory were excluded.

Ethical approval

Ethical approval was sought for and obtained from the ethical and research committee of Kaduna State Ministry of Health.

Informed consent

A written informed consent was sought from each patient and all information kept confidential.

Sample collection

A total volume of 2.5ml blood sample was collected from the ante-cubical vein into 0.04ml ethylene diamine tetra acetic acid (EDTA) bottle. Thick and thin blood films were prepared, RDT was carried out. The blood films were allowed to air-dry and stored safely until processed. In case of any delay, the EDTA samples were stored at 4^{o}c.

Laboratory analysis

Malarial parasitaemia was determined through microscopic examination of stained thin and thick films according to standard procedures [23] and detection of malaria parasites antigen using RDT kits. The detection of the malaria parasite antigen was carried out with the aid of BIO LINE SD Malaria Antigen P. *falciparum* manufactured by SD Standard Diagnostics, Inc., 156- 168 Hagal=dong, Giheung-gu, Yongin-si, Kyongi-do, Korea according to manufacturer's instructions.

Estimation of parasites density

The malaria parasite numbers/µl of blood was estimated by counting the number of malaria parasites seen against 100 white blood cells (WBC) in a Giemsa stained thick film and result expressed using this following formula described by [23]

$$\text{No of parasites/µl of blood} = \frac{8000 \times \text{No of parasites counted against 100 WBC}}{100}$$

STATISTICAL ANALYSIS

The data collected was analyzed using statistical package for social science software (SPSS V.23) in a 2 × 2 contingency tables and McNemar test [17]. The kappa coefficient as a measure of agreement for qualitative items was determined to confirm the consistency of the results among the diagnostic tools. The kappa values were used to categorize the strength of agreement between the microscopic examination and RDT. Values were interpreted with the

Landis and Koch classification: poorly correlated (<0), slightly correlated (0-0.2), fairly correlated (0.21-0.40), moderately correlated (0.41-0.60), substantially correlated (0.61-.80), and perfectly correlated (0.81-1.0) [17, 24]. Chi-square and student t-test were used to determine the variable. Value of $p< 0.05$ was considered significant. The key variables considered were:

$$\text{Sensitivity (\%)} = \frac{\text{Number of true positives (TP)}}{\text{Number of True positives (TP) + number of false negatives (FN)}} \times 100$$

$$\text{Specificity (\%)} = \frac{\text{Number of True negatives (TN)}}{\text{Number of True negatives (TN) + number of false positives (FN)}} \times 100$$

$$\text{Positive predictive value (PPV \%)} = \frac{TP}{TP + FP} \times 100$$

$$\text{Negative predictive value (NPV \%)} = \frac{TN}{TN + FN} = \times 100$$

$$\text{False positive rate (FPR \%)} = \frac{FP}{FP + TN} \times 100$$

$$\text{False negative rate (FNR \%)} = \frac{FN}{FN + TN} \times 100$$

$$\text{Efficay / Accuracy} = \frac{TP + TN}{TP+TN+FP+FN} \times 100$$

$$\text{Reliability} = \frac{TP \times TN - FP \times FN}{(TP + FN)(TN + FP)}$$

RESULTS

Out of the 300 patients examined, 76 (25.3%) were parasitaemic by microscopy with an average parasite density of 240/µl of blood. The rapid diagnostic tests (RDTs) detected antigenaemia in 81 (27%) patients (Table 1).

Tabl-1: Prevalence of malarial parasites among patients in Kaduna State based on the malaria diagnostic methods used

Test method	number examined	Number positive (%)
RDT	300	81(27.0)
Microscopy	300	76(25.3)

With respect to study facility, the highest prevalence rate of 38.0% was obtained in patients attending Gwamna-Awan General Hospital, Kakuri, while the least (16.0%) was recorded in patients who attended General Hospital, Sabon-Tasha. However, Yususf Dan Tsoho General Hospital, Tudun-Wada recorded 22.0%. The difference was not statistically significant (P = 0.625) as shown in Table 2.

Table-2: Prevalence of malarial parasites among patients in Kaduna State by study facility.

Study facility	Number examined	Number positive (%)	Number negative (%)
Kakuri	100	38 (38.0)	62 (62.0)
Tudun-Wada	100	22 (22.0)	78 (78.0)
Sabon-Tasha	100	16 (16.0)	84 (84.0)

* = significant association exists at $p \leq 0.05$

In relation to demographic and socio-economic factors, patients whose age were <10 years had the highest prevalence of 31.3% while those within the age group of 50 – 59 years had the least prevalence of 10.5%.The difference was not statistically significant (χ^2 = 4.380, p = 0.625). Based on gender, the female patients had a higher prevalent rate of 29.1 % while the male had a lower rate of 20.0%. However, this difference was not statistically significant (χ^2 = 3.222, p = 0.73).

With respect to marital status, the prevalent rate was highest amongst the married patients (27.7%) while widow had the least prevalence of 22.2%. The difference was not statistically significant (χ^2 = 0.767, p = 0.682). Based on educational status, those who are in the nursery and Islamic education category had the highest prevalence of 40.0% each whereas those who had no formal education recorded a prevalence of 19.0%. There was no statistically significant difference (χ^2 = 4.111, p = 0,534). With respect to occupation, patients in the business group had the highest prevalence of 30.1% while the lowest prevalent rate of 10.3% was recorded amongst the unemployed group. The difference was not statistically significant (χ^2 = 6,625, p = 0.357).

Table-3: Prevalence of malarial parasites among patients in Kaduna in relation to demographic and socio-economic factors

Factors	No Examined	No Negative (%)	No Positive (%)	χ^2	P-value
Age group					
< 10	32	22(68.8)	10(31.3)	4.380	0.625
10 – 19	64	46(71.9)	18(28.1)		
20 – 29	64	51(79.7)	13(20.3)		
30 – 39	64	46(71.9)	18(28.1)		
40 – 49	44	33(75.0)	11(25.0)		
50 – 59	19	17(89.4)	2(10.5)		
≥ 60	13	9(69.2)	4(30.8)		
Sex					
Male	125	100(80.0)	25(20.0)	3.222	0.073
Female	175	124(70.9)	51(29.1)		
Marital status					
Married	141	102(72.3)	39(27.7)	0.767	0.682
Single	150	115()76.7	35(23.3)		
Widowed	9	7(77.8)	2(22.2)		
Educational status					
None	21	17(80.9)	4(19.0)	4.111	0.534
Nursery	5	3(60.0)	2(40.0)		
Primary	48	37(77.1)	11(22.9)		
Secondary	101	70(69.3)	31(30.7)		
Tertiary	120	94(78.3)	26(21.7)		
Islamic	5	3(60.0)	2(40.0)		
Occupation					
Unemployed	32	28(87.5)	4(10.3)	6.625	0.357
Student	110	78(70.9)	32(29.1)		
Civil servant	55	43(78.2)	12(21.8)		
Business	83	58(69.9)	25(30.1)		
Artisan	4	3(75.0)	1(25.0)		
Farmer	8	7(87.5)	1(16.7)		
Retiree	5	4(80.0)	1(20.0)		
Settlement type					
Rural	21	17(80.9)	4(19.0)	0.472	0.492
Urban	279	207(74.2)	72(25.8)		
ITNs use					
Yes	177	126(70.8)	51(28.8)	2.764	0.096
No	123	98(79.7)	25(20.3)		

* = significant association exists at p ≤ 0.05

In relation to settlement type, patients who lived in the urban areas had the highest prevalent rate of 25.8% while those in the rural areas had the least of 19.0. There was no statistically significant difference in the result (χ^2 = 0.472, p =

0.492). With respect to the use of insecticide treated bed nets (ITNs), patients who sleep under ITNs had the highest prevalence of 28.8% compared to those who do not sleep under ITNs with prevalent rate of 20.3%. However, the difference was not statistically significant ($\chi^2 = 2.764$, $p = 0.096$). They are as represented in Table 3.

On the whole, 70 (23.3%) patients were positive by both RDT and microscopy (True positives) while 213 (71%) were negative by both methods (True negatives). The result also showed that 11 were negative by microscopy but positive for RDT (False positives) whereas 6 were negative by RDT but positive by microscopy (False negatives). There was statistically significant agreement between the two diagnostic methods ($k = 0.853$; $p < 0.001$) (Table 4).

Table-4: Contingency table of comparison for the RDT with microscopy

RDT	Microscopy		Total		Cohen's kappa coefficient	
	Positive	Negative			K	p-value
Positive	70	11	81		0.853	< 0.001*
Negative	6	213	219			
Total	76	224	300			

* = statistically significant agreement exists between microscopy and RDT at $p < 0.05$

The analysis of the performance of the RDT using standard format indicated that the RDT had a sensitivity of 92.1%, specificity of 87.3%, PPV of 86.4%, a NPV of 97.3%, a FPR of 4.9%, FNR of 2.7%, an efficacy of 94.3% and a reliability of 87.2% (Table 5).

Table-5: Diagnostic performance of the RDT

Indices	Value
True positives	70
True negatives	213
False positives	11
False negatives	6
Sensitivity (%)	92.1
Specificity (%)	87.3
Negative predictive value (NPV)(%)	86.4
Negative predictive value (NPV)(%)	97.3
False positive rate (FPR) (%)	4.9
False negative rate (FNR) (%)	2.7
Efficacy (%)	94.3
Reliability (%)	87.2
Kappa (k) value	0.853

DISCUSSION

Peripheral blood film microscopy and RDT showed *P. falciparum* was the only species found in positive slides and RDT cassettes. The detection of *P. falciparum* as the sole *Plasmodium* species identified in this study is not surprising. This finding is consistent with other reported studies where *P. falciparum* was either the predominant or only *Plasmodium* species detected [25, 24]. This is in agreement with the fact that *P. falciparum* has been the most endemic species of malaria parasites in sub-saharan Africa [6].

SD Bioline malaria Ag p.f/pan test kit detected more antigenaemia (27%) than microscopic parasitaema (25.3%). This is in agreement with the report of [24] that had RDT antigenaemia of 8.6% more than microscopic parasitaemia of 7.5%. Reason for this variation in positivity rate even though in the same region, may be attributed to the symptomatic subjects used in this study compared to their asymptomatic blood donor subjects. The higher positivity rate in RDT than microscopy may be attributable to cross-reactivity with heterophile antibodies [15] and persistence of residual antigens for weeks after treatment [16]. The same observation was reported by [17].

The low proportion of false positive and false negative tests is characteristic of a good diagnostic test. False positive tests are attributed to cross-reactivity with heterophile antibodies [15]. Also, the false negative RDT may be due to low parasitaemia as in the majority of cases or by interpreting the RDT before the test line has fully developed [16].

The prevalence of the parasite seen in Gwamna-Awan General Hospital, Kakuri could be attributable to environmental factor, attitude and lifestyle as well as the economic status of the study groups in this area. It was reported by [26] that incidence of malaria has been found significantly associated with some of these epidemiological factors.

With respect to age group, the highest prevalence was recorded among patients that were <10 years. This finding concurred with the reports of [24] though their subjects were in the age group of <20 years. This buttressed the affirmation that younger ones under 5years of age are more disposed to the infection than older persons in malaria endemic areas of sub-saharan Africa. These young individuals may become relatively protected against disease after repeated exposure to multiple malaria infections as they grow older [25].

The higher prevalence recorded for the female patients in this study, is in agreement with the findings of several other studies [27, 24]. This is also consistent with assertion that females are more vulnerable to this disease than male in Africa. Evidence from some countries indicates that restricted mobility of women may also impede their attendance at primary health care clinics for malaria testing thereby increasing their risk of infection [28]. There is a possibility that some of the females might be pregnant whose risk of infection increases due to changes in their hormone levels and immune system making them more susceptible to malaria [29].

The result of this study indicated a higher prevalence among married patients compared to the unmarried individuals. This is similar to that reported by [30, 24]. The reason for this present result could be by chance.

In this study, the business class had the highest prevalence. This may be due to their frequent business travelling tours that may take them to high risk areas and the fact that some businesses may extend to dark hours and in unscreened rooms which predispose them to contact with mosquitoes. It could also be by chance.

The result also revealed that prevalence was highest among urban dwelling patients. This finding is in agreement with the reports of [31, 24]. It has been shown that high population densities and possible lower immunities may result in more disease impact in urban setting [32].

The high prevalence of infection among patients who use insecticide treated bed net could be attributable to factors such as presence of pyrethroid resistant *Anopheles* in the area, inconsistent use of ITNs, proportion of those using ITNs in the study area, incorrect hanging of ITNs, use of expired ITNs and the biting behaviour of some *Anopheles* mosquitoes [5]. These are factors that could undermine effectiveness of ITNs in the study area. More than half of the people in a community must use ITNs for it to be effective [5]. Promoting the culture of appropriate net use based on effective education, promotion and marketing is essential to the success of the use of ITNs as far as public health is concerned [33].

The sensitivity of 92.6% reported in this study is a little lower than the recommended value of >95% [13, 14]. However, this sensitivity is higher than the 42.5% and 77.8% reported elsewhere in Nigeria by [34] and [24] respectively. The high specificity of 95.3%, efficiency of 94.6% and substantial correlation ($k = 0.853$, $p = 0.001$) with reference method makes RDT a potential tool for detection of *P. falciparum* in malaria endemic areas. The storage temperature the RDT product is subjected to by marketers could cause low sensitivity. Exposure of RDT kit to high temperature has been implicated as a possible cause of poor performance in the tropics. Denaturation of antibodies in the test membrane can impair binding to the target antigen at high temperature. Heat can also cause damage to the nitrocellulose membrane forming the strip thus changing its flow characteristics or causing the antibody to detach from the membrane [18]. Furthermore, it could be as a result of variation in the histidine rich-protien 2 (HRP 2) genotype of *P. falciparum* [19].

CONCLUSION/RECOMMENDATION

In conclusion, we demonstrated that quality RDT (SD BIO LINE) kit is reliable, rapid, easy to use and simple to interpret. The RDT is a valuable tool to complement microscopy in places where inexperienced laboratorians are lacking and facilities for microscopy are poor. In line with this, we concur to earlier recommendations for the use of the RDT kit in the management of febrile patients in malaria endemic regions and for epidemiological studies. RDT cost effectiveness notwithstanding; its advantages over microscopy and clinical diagnosis offer a more promising strategy to deal with increasing costs of therapy driven by drug resistance. Further studies will be required to assess probable way factors like bad storage temperature and others could be eliminated. This is with the view to enhance RDT kit sensitivity beyond the >95% recommendation by World health organization for higher performance.

REFERENCES

1. WHO. Malaria fact sheet. WHO, Geneva. Switzerland. Retrieved April, 2016 from *http://www.who.int/mediacentre/factsheets/fs094/en/*. 2016.
2. Owusu-Ofori AK, Betson M, Parry CM, Stothard JR, Bates I. Transfusion-transmitted malaria in Ghana. Clinical infectious diseases. 2013 Mar 5;56(12):1735-41.
3. Gruell H, Hamacher L, Jennissen V. On taking a different route: An unlikely case of malaria by nosocomial transmission. *Clinical Infectious disease*. 2017; 65:1404.
4. *Mace KE, Arguin PM. Malaria Surveillance-United States, 2014. MMWR Surveillance Summit. 2017; 66:1*

5. Centers for Disease Control and Prevention (CDC). Impact of Malaria, Available at *http://www.cdc.gov/ malaria/malaria_worldwide/impact.html*>. 2018.
6. Center for disease control and prevention, (CDC). Malaria facts. Retrieved April 15, 2016 from *http://www.cdc.gov/malaria/about/facts.html.* 2016.
7. Federal Republic of Nigeria. (FRN). Nigeria malaria fact sheet. Retrieved Dec, 2011 from *http:/photos.state.gov/libraries/nigeria/231771/Public/Dec-MalariaFactSheet2.pdf.* 2011.
8. Bhandari PL, Raghuveer CV, Rajeer A, Bhandari PD. Comparativestudy of peripheral blood smears, quantitative buffy coat and modified centrifuged blood smears in malaria diagnosis. *Indian Journal of Pathology.* 2008; *51: 108-112.*
9. *WHO. Guidelines for the Treatment of Malaria. 2nd ed. Geneva: World. Retrieved March 2010 from http://www.evidence4health.org/about-ehcrc/case-studies/who-guidelines. 2010.*
10. Moody A. Rapid diagnostic tests of malaria parasites. *Clinical Microbiology Review.* 2002; *56: 66-78.*
11. Achan J, Tibenderana J, Kyabayinze D, Mawejje H, Mugizi R, Mpeka BT. Case management of severe malaria–a forgotten practice: experiences from health facilities in Uganda. *PLoS one.* 2011; *6:e17053.*
12. *Batwala V, Magnussen P, Nuwaha F. Comparative feasibility of implementing rapid diagnostic test and microscopy for parasitological diagnosis of malaria in Uganda. Malaria Journal. 2011;10:373.*
13. WHO. New perspectives in malaria diagnosis. WHO Geneva, Switzerland.Retrieved May, 2000 from *http://www.who.int/malaria/publications/atoz/who*, 2000.
14. *Wongsrichanalai C, Barcus MJ, Muth S, Sutamihardja A, Wernsdorfer WH. Review of malaria tools: microscopy and rapid diagnostic test (RDT). American Journal of Tropical Medicine and Hygiene. 2007; 77:119–127.*
15. Moody AH, Chiodini PL. Non-microscopic method for malaria diagnosis using OptiMAL IT, second-generation dip-stick formalaria p L DH antigen detection. *British Journal of Biomedical Science*. 2002; 59:228–231.
16. Heutmekers M, Gillet P, Cnops L, Bottieau E, Van Esbroeck M, Maltha J, Jacobs J. Evaluation of the malaria rapid diagnostic test SDFK90: Detection of both PfHRP2 and Pf-pL DH. *Malaria Journal*. 2012; 11:359.
17. Pembele G N, Lázara R R, Jorge F. Detection and Species Identification of Malaria Parasites by Nested-PCR: Comparison with Light Microscopy and with SD BIOLINE Malaria Ag Test in Luanda, Angola, *International Journal of tropical disease and Health.* 2015*; 10(1): 1-13.*
18. Chiodini PL, Bowers K, Jorgensen P, Barnwell JW, Grady KK. The heat stability of *Plasmodium* lactate dehydrogenase-based and histidine-rich protein 2-based malaria rapid diagnostic tests. *Transaction Royal Society of Tropical Medicine and Hyg*iene. 2007; 101: 331-337
19. Ayogu EE, Ukwe CV, Nna EO. Assessing reliability of microscopy and rapid diagnostic tests in malaria diagnosis in areas with varying parasite density among older children and adult patients in Nigeria. *Journal of Postgraduate Medicine*. 2016; 62(3):150-156
20. Encyclopedia Britannica. Kaduna State, Nigeria. Available at *www.britannica.com/place/Kaduna-Nigeria.* 2016.
21. Naing L, Winn T, Rausli BN. Practical issues in calculating the sample size for prevalence studies. *Archive of orofacial science.* 2006; *1: 9-14*
22. Osagiede ON, Maikaje DB, Umar YA. The prevalence of malaria in patients from selected Local Government Areas of kaduna State, North-west, Nigeria. *Nigerian Journal of Parasitolog.* 2017; 38 (1): 12
23. Cheesbrough M. District Laboratory Practice in Tropical Countries Part I, 2nd edition update. Cambridge University Press, *UK.* 2010; *PP 239-258.*
24. Kanyi OI, Ajayi MB, Ezeugwu SM, Afocha EE, iwalokun b. Comparison of rapid diagnostic test (rdt), polymerase chain reaction (pcr) and microscopy methods in the diagnosis of malaria among airport workers in lagos. Nigerian Journal of Science and Environment. 2016;13:1.
25. Umaru ML, Uyaiabasi GN. "Prevalence of Malariain Patients Attending the General Hospital Makarfi, Kaduna – State, North-Western Nigeria."*American Journal of Infectious Disease Microbiology*. 2015; 3(1):1-5.
26. Sultan M, Sheikh N, Mahmud RA, Jahir T, Islam Z, Sarker AR. Prevalence and associated determinants of malaria parasites among Kenyan children. *Journal of Tropical Medicine and Health.* 2017; 45:25
27. Oche AO, Aminu M. The prevalence of malarial parasitaemia among blood donors In Ahmadu Bello University Teaching Hospital, Shika, Zaria, Nigeria. *Nigeria Journal of Medicine*. 2012; 21(4):445-449.
28. Lampietti J. Gender and preferences for malaria prevention in Tigray, Ethiopia. Policy and Research Report on Gender and Development. *The World Bank Development Research Group/Poverty Reduction and Economic Management Network*: 1999; 3.
29. Kourtis AP, Read JS, Jamieson DJ. Pregnancy and infection. New England Journal of Medicine. 2014; 370 (23) : 2211 – 2218.
30. Alli JA, Okonkwo IO, Abraham OA, Kolade AF, Ogunjobi PN. A survey of Blood Parasites (Plasmodium, Microfilaria, HIV, HBsAG, HCV) antibodies in Prospective Nigerian Blood Donors. *Research Journal of Medical Science*. 2010; 4(4):255-275
31. Okonkwo IO, Adejuwon, OA, Okerentugba PO, Innocent-Adiele HC. CirculatingPlasmodium falciparum and HIV ½ as Co-infections among Blood Donors inIbadan, Southwestern, Nigeria. *Nature and Science*. 2012; 10(9):42-47.

32. Udeze AO, Nwokocha EJ, Okerentugba PO, Anabinjuwon II, Okonkwo IO. Asymptomatic Plasmodium Parasitaemia in Ilorin, North Central Nigeria. *Nature and Science.* 2013; 11(10):24-8.
33. Guillet P, Alnwick D, Cham MK, Neira M, Zaim M, Heymann D. Long- lasting treated nets: a breakthrough in malaria prevention. *Bulletin World Health Org*anization. 2001; 79:983-988.
34. Oyeyemi O, Akinwale T, Ogunlade F, Isaac OO. Comparative assessment of microscopy and rapid diagnostic test (RDT) as malaria diagnostic tools. *Research Journal of Parasitology.* 2015; *10: 120-126.*