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Research Article

Utilization of Saliva in Diagnosis of *Plasmodium falciparum* Using Rapid Diagnostic Tests Kits in Dutsin-ma, Katsina, Nigeria

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ABSTRACT

A diagnostic technique that is easy to perform, sensitive and also specific is critically essential in any disease control plan. This study aimed to determine the viability of utilizing saliva in diagnosis of *Plasmodium falciparum*, using rapid diagnostic tests (RDT) kits in Dutsin-ma, Katsina, Nigeria. Blood and saliva samples from suspected malaria patients were obtained and analysed for the detection of *Plasmodium falciparum* histidine rich protein 2 using pfhrp2 antigen detection kits (first response and arkray). Malaria parasitaemia count was also determined by microscopy. The sensitivities and specificities of both RDT-blood and RDT-saliva were compared. The result of 376 suspected malaria patients that provides both blood and saliva samples for this study revealed a prevalence rate of 54.3% *Plasmodium falciparum* infection by RDT-blood. The sensitivities of RDT-blood and RDT-saliva are 53.57% and 10.36% respectively. Also, it was observed that RDT-SALIVA missed a lot of positive cases especially in patients with low parasitaemia count confirmed by microscopy.

Keywords: Diagnosis; Microscopy; *Plasmodium falciparum;* Prevalence; RDT Kits; Saliva; Sensitivity; Specificity

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INTRODUCTION

Malaria is a parasitic disease caused by *Plasmodium*, with an estimated 216 million cases annually. (World Health Organization 2012). WHO latest report on malaria estimated number of malaria death at 608,000 with 95% in WHO African region (WHO. 2023).

Plasmodium falciparum remains one of the most important infectious diseases in sub-Saharan Africa (SSA). Despite reduction in malaria morbidity and mortality since the beginning of the third millennium, 214 million people (88% living in SSA) acquired malaria and 438,000 people (90% from SSA) died from malaria in 2015 (WHO, 2015). *Plasmodium falciparum* is estimated to account for more than 95% of malaria cases in Nigeria. Interventions, such as insecticide-treated nets, have played a great role to the decrease in the number of malaria cases and deaths. Early diagnosis and prompt treatment of cases are essential for addressing the global burden of malaria (Aninagyei *et al*., 2020). The ideal diagnostic method that would be most beneficial for eliminating malaria needs to be rapid, simple to perform, inexpensive, sensitive, accurate and non-invasive.

Most common reasons for malaria death include incorrect, delayed, or unavailable diagnosis (Poschl *et al.,* 2010). WHO recommended that malaria infection must be parasitologically confirmed before initiating treatment, since clinical signs may be misleading (WHO 2012).

Currently available diagnostic methods for malaria include the identification of malaria parasites or

parasite proteins in blood by microscopy and rapid diagnostic tests (RDT) and parasite DNA by polymerase chain reaction (PCR). Microscopic examination of Giemsa-stained thick blood film is the diagnostic gold standard, but even trained microscopists routinely detect only 50–100 parasites or more/microlitre of peripheral blood. Joanny *et al*. (2014) thus missing infected individuals with very low parasitaemia. Despite differences in their procedures and performances, microscopy, RDT, and nested PCR (nPCR) for malaria diagnosis share a common problem, that is, the requirement of blood samples. This invasive procedure of using hypodermic needle can cause adverse effects such as pain or bruise at site of puncture, anxiety and even fainting, nerve damage and haematoma in patients. Again, poor infection-control practice can lead to microbial infection and both patients and health workers can be exposed to blood-borne infection from infected individuals (Aninagyei *et al*., 2020).

Most Previous studies that identified pfhrp2 in salivary samples uses more sensitive diagnostic techniques (enzyme immuno-assays and fluorescent immuno-assays). These techniques are costly and are only available in reference and specialized laboratories. Malaria is mostly endemic in rural areas where these techniques and technical expertise that can operate them are lacking.

The above listed demerits of invasive procedure have led to suggestions and evaluation of non-inversive diagnosis procedures in some studies. Saliva is one of the most popular alternative specimens to blood, for diagnosis of malaria. It doesn't require invasive procedure, it is simple to perform, safe, painless, no special equipment needed and can be done by individuals with limited training including patient themselves, and it also allow for multiple collection even outside the laboratory (Aninagyei *et al.,* 2020).

Aninagyei *et al*. (2020) used RDT kit to detect pfhrp2 in saliva patients in Ghana west municipal and recorded a sensitivity rate of 57.0%. This study was therefore design to detect pfhrp2 specific antigen in Salivary specimen of suspected malaria patients using readily available RDT kits.

MATERIALS AND METHODS

Study site

This study was conducted in Dutsin-ma, Katsina State of Nigeria. Dutsin-Ma Local Government Area is located in Katsina West where it occupies an area of 552.323Km^2. It has a population of 169,829 inhabitants according to the 2006 census (FGN Census, 2012).

Medical out Patients who visited the above-mentioned health centers who showed clinical signs of malaria including adults and children of both genders were included in this study. Those that are unable to provide saliva samples were excluded.

Sample size determination

The sample size was determined using the formula - $n=z^2\ p(1-p)/d^2$ (Aninagyei *et al.,* 2020). Where;

N = sample size

p = malaria prevalent in Dustin-Ma (42.86%) (Amiru *et al*., 2024)

z= confident level at 95%. (Standard value of1.96) d= margin error at 5% (standard value of 0.05). The sample size was calculated to be 376.

The list of clinically suspected patients visiting the laboratory departments of the health centres for diagnosis were used to choose participants using a rigorous random selection technique.

Blood and saliva samples collection

Blood samples were collected by venipuncture into EDTA tubes by trained laboratory technicians at the health centres before analysis. Saliva samples were also obtained from the same patients in urine sample bottles before analysis. No special storage procedure was used as samples were analysed immediately after collection.

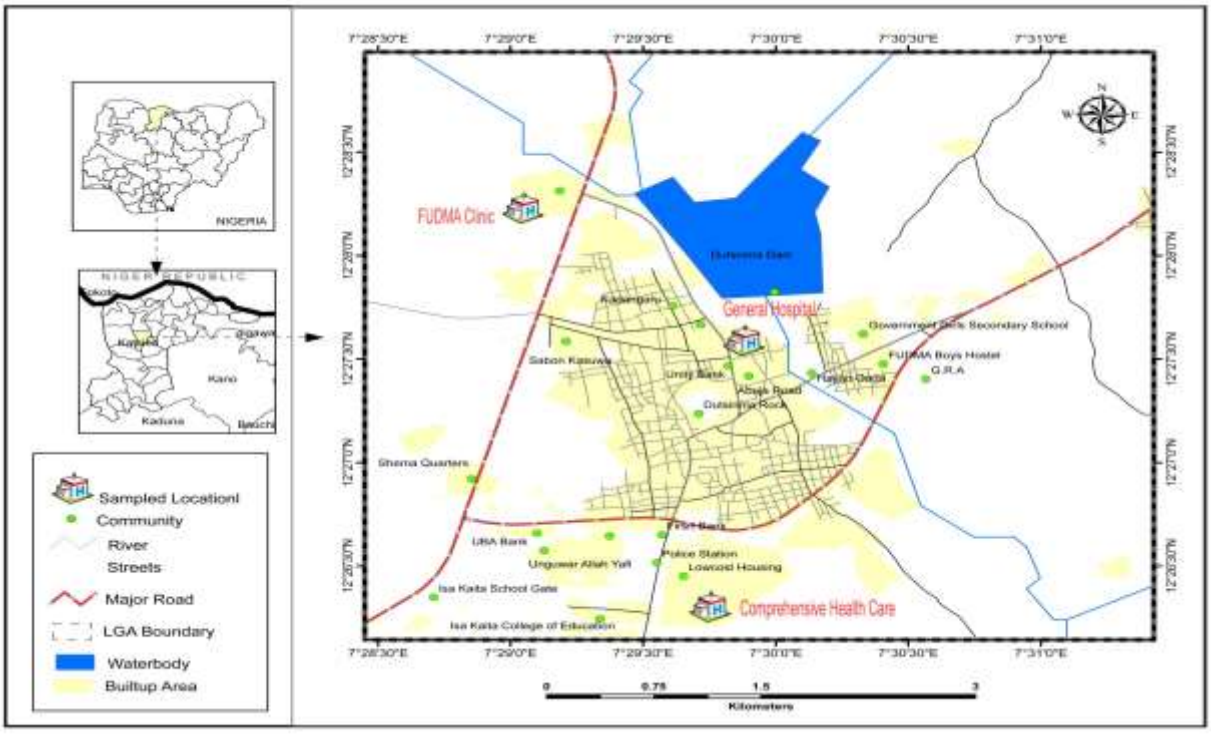

Figure 1: Map of Dutsinma Local Government Area showing the study area (Source: GIS FUDMA, 2024)

Laboratory Analysis

Malaria microscopy

To quantify malaria parasites, a thick blood smear was prepared and air dried. The air-dried films were stained with 10% Giemsa for 10 minutes, for parasites identification and parasitaemia count (WHO, 2019).

Malaria RDT for blood and saliva samples

Plasmodium falciparum specific antigen pfhrp2 was detected using RDT kits (first response and arkray). Blood RDT was performed in accordance to manufacturer's instructions. RDT saliva was performed with the same kits with some modification. Test results were read after 20 minutes.

Data Analysis

Data were entered into excel 2006 and was statistically analysed using special package for social sciences (SPSS) version 23. Descriptive statistics was used to analysed demographic characteristics and prevalence of malaria among participants. Chi square was used to determine relationship between variables and sensitivity test was used to determine the sensitivities and specificities of variables.

RESULT

Demographic characteristics

Table 1 provides a sociodemographic breakdown of suspected malaria patients by gender and age. The sample consists of 114 males (30.30%) and 262 females (69.70%). The age distribution shows that the highest number of suspected malaria cases occur within the age group 21-30 year (98 cases, 26.1%), followed by the 1-10 years age group (75 cases, 19.9%). 31-40 years (65 cases, 17.3%), then 11-20 years (62 cases, 16.5%). The age group 41-50 recorded (47 cases, 12.5%). The lowest prevalence occurs among the age group 51 years above (29 cases, 7.7%).

Prevalence of malaria

Table 2 presents information about prevalence of *Plasmodium falciparum* within the study population. Two hundred and four patients tested positive (54.3.%), while 172 tested negative (45.7%). The overall prevalence rate among studied population was 54.3%.

Table 3 presents the distribution of saliva results positive cases with correspondent parasitaemia count by microscopy. Out of 29 saliva samples that indicate a positive result for the detection of *Plasmodium falciparum*, twenty-seven (27) cases were confirmed by microscopy to be +++ and 2 cases was confirmed to be ++++. Also 9 cases of microscopy results that shows parasitaemia count of +++ indicate a negative case by saliva-based RDT. However, the relationship between variables was statistically significant as p value is less than 0.05.

Table 1: Distribution of participants by Gender and Age interval

Gender	Frequency	Percentage
Male	114	30.30
Female	262	69.70
Age (Years)		
1-10	75	19.9
11-20	62	16.5
21-30	98	26.1
31-40	65	17.3
41-50	47	12.5
51 Above	29	7.7
Total	376	100

Table 2. Prevalence of *Plasmodium falciparum* among participants

Test Result	Frequency	Prevalence %
POSITIVE	204	54.3
NEGATIVE	172	45.7
TOTAL	**376**	**100**

Table 3: Distribution of RDT saliva positive cases by parasitaemia count

Parasite count/µl of blood	Positive	Negative	Total	Chi Square	P Value
Negative	0	96	96	277.471	0.000
Scanty	0	38	38		
+	0	138	138		
++	0	66	66		
+++	27	9	36		
++++	2	0	2		
Total	29	347	376		

Table 4 presents the results of the comparison of RDT Blood with RDT saliva for the detection of *Plasmodium falciparum*. Out of total study population (376), two hundred and four (204) samples are positive by blood RDT and 172 were negative. However, saliva-based RDT detected only 29 cases with positive result and 347 negative cases. The association was statistically significant as p value is 0.000.

Table 5 presents the results of the comparison of diagnostic performance between RDT blood and RDT saliva. The blood sample result present high sensitivity rate (53.57%) in comparison to saliva sample whose rate is low (10.36%). In term of specificity, the saliva sample has specificity rate of 100% while blood sample shows also high specificity of 93.75%. The blood sample recorded a high accuracy rate (63.83%) compared to accuracy rate recorded by saliva sample (33.24%).

Table 4: Comparison of saliva and blood sample results for *Plasmodium falciparum* detection

RDT (SALIVA)	RDT (BLOOD)		Total	Chi Square	P value
	Positive	Negative	Total		
Negative	175	172	347	**26.494**	**0.000**
Positive	29	0	29		
Total	204	172	376		

Table 5: Comparison of diagnostic performance between blood and saliva samples

Diagnostic Test	RDT saliva	RDT blood
Sensitivity (%)	10.36	53.57
Specificity (%)	100	93.75
Accuracy (%)	33.24	63.83

DISCUSSION

The gender difference recorded in this study may be influenced by several factors, including varying exposure risks, differences in healthcare-seeking behaviour, and potential biological susceptibility. Studies have shown that women are more likely to seek medical attention for symptoms consistent with malaria (Snow *et al.*, 2022; Koekemoer *et al*., 2021).

The age group however could be due to rigorous random sampling method that might have favour the age group (21-30), while the 1-10 age group aligns with global data indicating that children under five are particularly vulnerable to malaria due to their developing immune systems and the lack of prior exposure to the parasite (WHO, 2023). The decline in cases with increasing age reflects the development of partial immunity over time, as seen in similar studies conducted in malaria-endemic regions (Smith *et al.*, 2022; Amek *et al.*, 2023). Notably, a study by Amek *et al.* (2023) in Kenya found that the incidence of malaria decreases significantly with age, with adults exhibiting lower parasite densities and fewer clinical symptoms compared to children.

The prevalence rate recorded in this study was higher compared to that of Amiru *et al*. (2024) who recorded a prevalence of 42.86% at the same region. The study was similar to the result of Aninagyei *et al*. (2020) who recorded a similar prevalence of 53.9% in Ghana. This varies from the records of Mac *et al.* (2019) who recorded an overall prevalence rate by RDT (44.9%) in Abuja and central states. Hamidu *et al*. (2017) also recorded a less prevalence of 19.52% in North-western states using RDT.

The distribution of saliva results positive cases in respect to parasite density has shown that saliva-based RDT was only effective in detecting high-density infections (+++ and ++++), but their sensitivity dropped significantly at lower parasite densities (Aninagyei *et al.*, 2020). This is particularly concerning given that low-density infections can still contribute to the transmission of malaria, especially in regions aiming for elimination (Okell *et al.*, 2022). Gbotosho *et al*. (2010) also reported a similar case in their findings. This differ from the findings of Aninagyei *et al.* (2020) who's findings revealed that all cases with higher parasitaemia count are confirmed positive by saliva-based RDT. The disparity however could be explained by the sensitivities of RDT kits used and procedures adopted.

In comparison of RDT Blood with RDT saliva for the detection of *Plasmodium falciparum*. A specie specific RDT kits meant to detect *Plasmodium falciparum* histidine rich protein 2 (*pf*hrp2) in a whole blood sample was used for both blood and saliva samples. The results obtained indicate that the saliva test misses almost 175 positive cases detected by RDT blood. The differences in detection capability observed could be explained by the kits being a blood based and also the quality nature of the kits. The saliva-based diagnosis in this study has indicate very low sensitivity rate (10.36%). This means that the result fails to detect many actual positive *Plasmodium falciparum* positive cases leading to high false negative cases. The specificity rate was 100%, meaning the saliva-based test perfectly identifies all true negative cases confirmed by blood RDT correctly. The overall accuracy is 33.24%. This differs from the findings of Aninagyei *et al.* (2020) who recorded a higher sensitivity of 57.0% and an accuracy of 78.6% for the saliva-based result using SD Bioline RDT kit. The specificity result was however similar with Aninagyei *et al*. (2020) record of 100%.

To compare the performance between RDT blood and RDT saliva, the blood sample result present high sensitivity rate in comparison to saliva sample whose rate was low. This differs from the findings of Wilson *et al.* (2008) who recorded a high sensitivity of 43% for saliva using Enzyme-link immuno sorbet assay (ELISA) technique in Mali, Gbotosho *et al.* (2010) with sensitivity of 77.9% using optiMAL-IT dipstick kit in Nigeria. Mfuh *et al. (*2017) also reported a higher sensitivity rate for blood 100% compared to saliva 95% using Nested polymerase chain reaction (nPCR). These findings also differ from the findings of Ardin (2020) who recorded similar sensitivity rate for both blood and saliva (43.90%) using LAMP technique targeting 18S rRNA gene. Quattara *et al.* (2011) also recorded a high sensitivity rate for saliva-based result (97.2%) in Mali. This study also differs from that of Imboumy *et al. (*2023) who reveals a sensitivity rate of 100% for blood RDT and 22.86% for saliva-based RDT using optiMAL-IT Biorad kit after DNA extraction and GAPDH gene amplification using nqPCR targeting STEVOR gene. It also differs from Aninagyei *et al*. (2020) record of 57.0% sensitivity for saliva-based RDT using SD-Bioline kit. In terms of specificity, the saliva sample have demonstrated a specificity rate of 100% while blood sample shows also high specificity of 93.75%. This shows that the saliva sample was able to detect all negative cases confirmed by blood sample. This is similar to the result of Aninagyei *et al. (*2020) who recorded 100% specificity for saliva-based result. It differs from that of Mfuh *et al. (*2017) who recorded a specificity of 93% and 87% for saliva and blood respectively. The findings also differ from that

of Ardin (2020) who reveals a specificity of 68.75% for saliva and 57.62% for blood. Quattara *et al.* (2011) also recorded a high specificity of 95.4% for saliva-based result. The blood sample recorded a high accuracy rate compared to accuracy rate recorded by saliva sample. This makes the blood test results more reliable than that of the saliva-based result. This is in contrast with the findings of Aninagyei *et al.* (2020) who recorded an accuracy rate of 76.8% for the saliva-based result. The differences recorded in this study compared to other studies could be explained by time-frame, quality of kit used, method of analysis as most uses a more sensitive molecular technique. Study Population also could be among the factors that trigger the differences as some used children below the age of five to increase their chances of recording more positive cases by saliva.

CONCLUSION

This study provides clear evidence on the demographic pattern, prevalence of malaria, and the comparative diagnostic performance of blood- and saliva-based rapid diagnostic tests (RDTs) for the detection of *Plasmodium falciparum* among suspected malaria patients. Findings from the saliva-based RDT analysis showed that positive saliva results were largely associated with high parasitaemia levels (+++ and ++++), while several microscopy-confirmed high-parasitaemia cases were not detected by saliva RDT. Although the association between saliva RDT results and parasitaemia count was statistically significant ($p < 0.05$), the low detection rate at lower parasite densities demonstrates the limited sensitivity of saliva-based RDTs. The comparison between blood and saliva RDTs further revealed a marked disparity in diagnostic performance. Blood-based RDT detected substantially more positive cases and demonstrated significantly higher sensitivity and overall accuracy compared to saliva-based RDT, despite saliva RDT exhibiting perfect specificity. These findings indicate that while saliva RDTs may reliably identify true negative cases, their low sensitivity severely limits their usefulness for routine malaria diagnosis. Given the superior detection capability, sensitivity and accuracy of blood-based RDTs, they should continue to be the preferred diagnostic method in areas where immediate and accurate malaria diagnosis is essential, there is need for more sensitization on the importance of integrated vector control strategy to avert mosquito bites and reduce prevalence rates of malaria.

This study has proved evidence that *Plasmodium falcifarum* antigen can be detected in the saliva samples of malaria patients despite demonstrating low sensitivity rate. More of this type of research is recommended to increase the viability of utilizing saliva in malaria diagnosis and subsequent innovation of saliva-based RDT kits.

Ethical clearance and participants consent: Ethical clearance was obtained from the Katsina State ministry of health under the Katsina health research ethical review committee with reference No.: MOH/ADM/SUB/1152/1/1018

A written consent was also sought from participants, parents or guardians of participants for children under 18 years before sample collection.

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