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Comparative Performance Evaluation of Symptomology, Point-of-Care Test and Microscopy in the Diagnosis of Malaria on Suspected Malaria Cases in Katsina, Nigeria

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Abstract

Malaria is the most dominant cause of human morbidity and mortality with huge medical, psychological and economic impact in Nigeria. Prompt and accurate diagnosis is one of the key components in the control of malaria disease. In Katsina State, clinical (symptomatic) diagnosis and *Pf* HRP-2 RDT are the two main methods routinely used for the diagnosis of malaria. Only tertiary, secondary and few primary hospitals employ microscopy in malaria diagnosis. This study was done to assess the performance of the clinical diagnosis, SD-BioLine (*Pf*HRP-2) rapid diagnostic tests (RDTs) and Microscopy in the diagnosis of Malaria disease in Katsina State**.** In this cross-sectional study, involving three hospitals, blood samples of 400 clinically suspected malaria patients were tested for malaria using microscopy with Giemsa-stained films and Rapid Diagnostic Test (RDT), using SD Bioline Pf HRP-2 kit. Malaria prevalence using microscopy was 29.8% (119/400). Pf HRP-2 RDT recorded lower sensitivity with a parasite prevalence of 23.8% (95/400). PfHRP-2 RDT was able to identify only patients infected with *P. falciparum* in comparison to microscopy that detected a prevalence of 6% of malaria infections other than *P. falciparum.* The research indicated that clinical diagnosis in Katsina state is not very effective in malaria treatment. PfHRP-2 RDT is not an ideal test kit, as there exist, other Plasmodium species, in Katsina State that can equally cause malaria infection.

Key words: Falciparum, Microscopy, Malaria, prevalence, PfHRP-2 RDT, Nigeria

Introduction

Malaria is one of the major public health problems. It is the most important cause of human morbidity and mortality with immense medical, emotional and economic effect in the world (*WHO, 2017; Beatrice et al., 2012*). Malaria occurs in nearly 100 countries worldwide. According to the World Malaria Report 2013, there were more than 200 million malaria cases in 2012. Between 2000 and 2013, the incidence rates of malaria fell by about 30% globally, and by 34% in Africa (Murray *et al*., 2014). As presented by WHO, malaria is a major public health problem in Nigeria where it accounts for more cases and deaths than any other country in the world (Sparkle, 2015; WHO, 2015). By 2010, malaria was said to be a risk for about 97% of Nigeria's population, the remaining 3% of the inhabitants live in the malaria free highlands (Olasehinde *et al*., 2015). It accounts for approximately 60% of outpatient visits and around 30% of hospitalizations among children under five years of age in Nigeria, contributing to an estimated 11% of maternal mortality (Olasehinde *et al*., 2015).

Traditional practice to diagnose malaria is empiric/syndromic diagnosis, where the diagnosis is made based on clinical history, signs, and/or symptoms. In many endemic areas that do not have sufficient diagnostic competency, patients with febrile illnesses are likely to get the diagnosis of malaria. Present methods of treatments are not promising towards eradication in many countries and the cost of maintaining these interventions has reached several billions of dollars each year (WHO, 2017).

Giemsa-stained microscopy and rapid diagnostic tests (RDTs) represent the two diagnostics most likely to have the largest impact on malaria control today. These two methods, each with peculiar strengths and limitations, together represent the best hope for accurate diagnosis as a key component of successful malaria control (CDC, 2016).

Clinical diagnosis and *Pf*HRP-2 Rapid diagnostic tests (RDTs) for malaria are considered for most patients in dispensaries, primary health care centers and some general hospitals in Katsina State, where there is a shortage of manpower and poor power settings to equitably handle microcopy. However, there is a very little evidence to guide decision-makers on the sensitivity and specificity of clinical diagnosis and the RDTs. There is a need to identify and establish the most effective and ideal method of malaria diagnosis to be adopted in Katsina State. This research was done to assess the performance of the clinical diagnosis, SD-BioLine (*Pf*HRP-2) rapid diagnostic tests (RDTs) and microscopy in the diagnosis of Malaria disease in Katsina State.

Materials and Methods

Study Area

Katsina state is located on the coordinates $12^{0}15^{`}$N and $7^{0}30^{`}$E. It has a population of 5,801,584 (2006 census) and covers an area of 24,192KM^{2}. It has an elevation of 519m above sea level, with an international boundary in the North to Niger Republic. It also shares border in the East with Kano and Jigawa States, in the West with Zamfara State and in the South with Kaduna State.

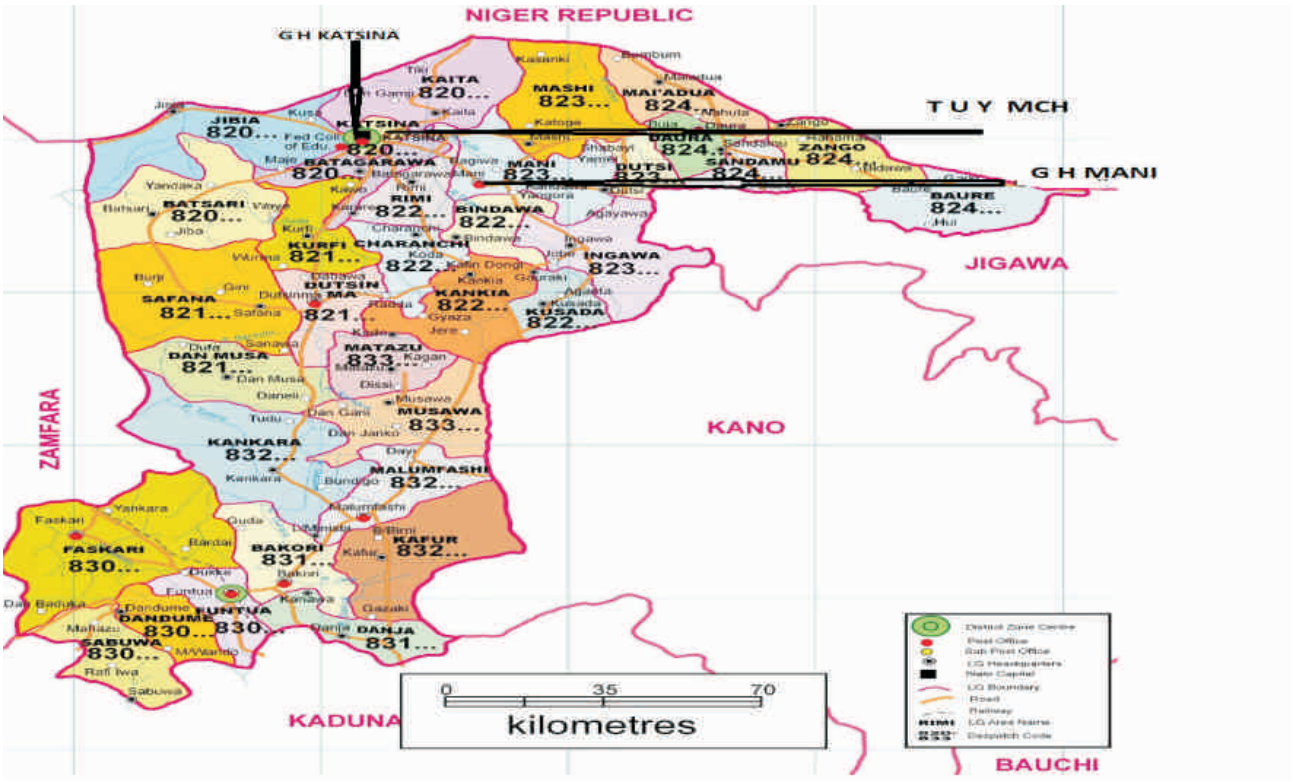

Figure 1: Map of Katsina State indicating sampling sites (maplandia.com)

Study design and sample size determination

This was a cross-sectional survey. The minimum sample size was determined using the Slovin's formula (Stephanie, 2018).

$$n = \frac{N}{1 + Ne^{2}}$$

Where, n = Number of samples required,
N = total population = 5,801,584
e = error tolerance, at confidence level of 95 percent, the margin error was 0.05

$$n = \frac{5{,}801{,}584}{1 + 5{,}801{,}584 \times 0.05^{2}} + 400$$

Study sites
The study was conducted in General Hospital Katsina, General Hospital Mani and Turai Umaru Yar`adua Maternal and Children Hospital (TUYMCH).

Purposively, based on the bed capacity of each of the sites, 164, 147 and 89 patients were recruited from General Hospital Katsina, Turai Umaru Yar`adua Maternal and Child Health Katsina and General Hospital Mani respectively.

Inclusion criteria: The inclusion criteria for enrollment included; clinically suspected malaria patients and patients referred to the laboratory for Malaria Parasite test.

Exclusion criteria: Non clinically suspected malarial patients were not enrolled, even if presented with malarial symptoms.

Ethical Clearance and Informed Consent
Ethical clearance was acquired from the ethical and human research committee of Katsina State Ministry of Health. Informed consent was obtained from all participating subjects in accordance with the standards of human experimentation and with the Helsinki Declaration of 1975, as revised in 2008 (Helsinki Declaration, 2008).

Samples collection
The samples were collected with the assistance of some Medical Laboratory Scientists and Medical Laboratory Technicians working at the collection sites. The method of sample collection employed was a venipuncture technique as described by Wobo *et al*. (2014).

Blood film preparation
Both thick and thin films smear were prepared adhering to the existing protocols (WHO, 2017; CDC, 2016; Cheesbrough, 2006).

Staining technique
The laboratory method employed for staining and identification of malaria parasites in the collected blood samples was done as previously described (WHO, 2015; Cheesbrough, 2006).

Microscopic examination
The dried, stained slides were examined for malaria parasites. Both the thick and thin smears prepared were examined microscopically under oil immersion with the (x100) objective (WHO, 2016).

Rapid Diagnostic Test using SD. BIO-LINE: Malaria *Ag Plasmodium falciparum*
A one step Malaria PfHRP-2 Antigen Rapid Test Kit was used for this research (being the one commonly used in Katsina State). Using a well-mixed blood sample, 0.005ml was dispensed into the round specimen well. Four (4) drops of the assay diluent were vertically dispensed into the square assay well. 15 – 30 minutes reaction was given before reading the results (WHO, 2019; SD, 2015).

Results
Clinical presentations of Subjects
The commonest clinical observations for malaria suspicion were fever, headache, raised body temperature, nausea, body pains, vomiting, shivering and anaemia (Table 1).

Clinical presentation of the study participants
From our findings, the most common symptoms presented by the patients were fever with about 90% (361/400) while anaemia were found to be the least symptoms observed by the patients with 22%, (86/400). Table 1 shows the breakdown of the other symptoms, and also in relation to the three study sites.

Prevalence of Malaria parasites
Using microscopy, there was an overall malaria prevalence of 29.75% (119/400. *Plasmodium falciparum* was the most diagnosed species accounting for 80% (95/119) of all the malaria positive slides. *P. vivax* was not detected from all the patient`s samples observed. A total of 16 patients, 4.0% (6 each from G.H.KTN, and TUY MCH, 4 from G.H Mani) were infected with *P. malariae* only. 8 (2.0%) patients (5 from G.H.KTN and 3 from TUY MCH) were microscopically diagnosed with *P. ovale* only. From the RDT data, out of the 400 participants, malaria prevalence was 23.75% (95/400), ranging from 14-31% for the three hospitals. All the samples that reacted positive to RDT (95) were also microscopically diagnosed with *P.*

falciparum (Figure 1). The detail of the above results is shown in table 2.

Figure 1 depicted the overall prevalence of malaria by symptoms, microscopy and RDT, and the results were compared.

Table 3 gave an insight on the relationship between malaria and anaemia (PCV ⩽ 29 %) in children (0-5 years) on clinically suspected malaria patients.

Figure 2 shows the prevalence of malaria in relation to age group in which the age group 6-12 had the highest with 44.4% and the age group 13-26 had the least with 17.6%.

Table 1: **Outcomes of clinical diagnosis from the study centres**

Variables	Total	Fever (%)	Headache (%)	Anaemia (%)	Raised Body Temperature (%)	Nausea (%)	Body Pains (%)	Vomiting (%)	Shivering (%)
GH KTN	164	155 (95)	132 (80)	24 (15)	64 (39)	33 (20)	126 (77)	24 (15)	44 (27)
GH MANI	89	89 (100)	77 (87)	30 (34)	55 (62)	33 (37)	66 (74)	44 (49)	39 (44)
TUY MCH	147	117 (80)	96 (65)	32 (22)	56 (38)	71 (48)	48 (33)	48 (33)	36 (24)
Total	400	361 (90)	305 (76)	86 (22)	175 (44)	137 (34)	240 (60)	116 (29)	119 (30)

Key:
GHKTN: General Hospital Katsina; TUYMCH: Turai Umaru Yar`adua Maternal and Children Hospital; GHMANI: General Hospital Mani

Table 2: RDT and microscopy findings among studied subjects

Location	RDT Positive Only (%)	Mixed Infections RDT + Other Spp. (Microscopy)		Single *Plasmodium spp.* Positive Slides			Positive Slides (%)	Negative Slides (%)	Total Samples
		RDT + P. M	RDT +P.O	P.M Only	P.O Only	P.F Only			
GHKTN	51(31)	1	0	6	5	51	62(38)	102(62)	164
GHMANI	24(27)	1	1	4	0	24	28(31)	61(69)	89
TUY MCH	20(14)	6	1	6	3	20	29(20)	118(80)	147
Total (%)	95 (23.75)	8 (2.0)	2 (0.5)	16 (4.0)	8 (2.0)	95 (23.75)	119 (29.75)	281 (70.25)	400

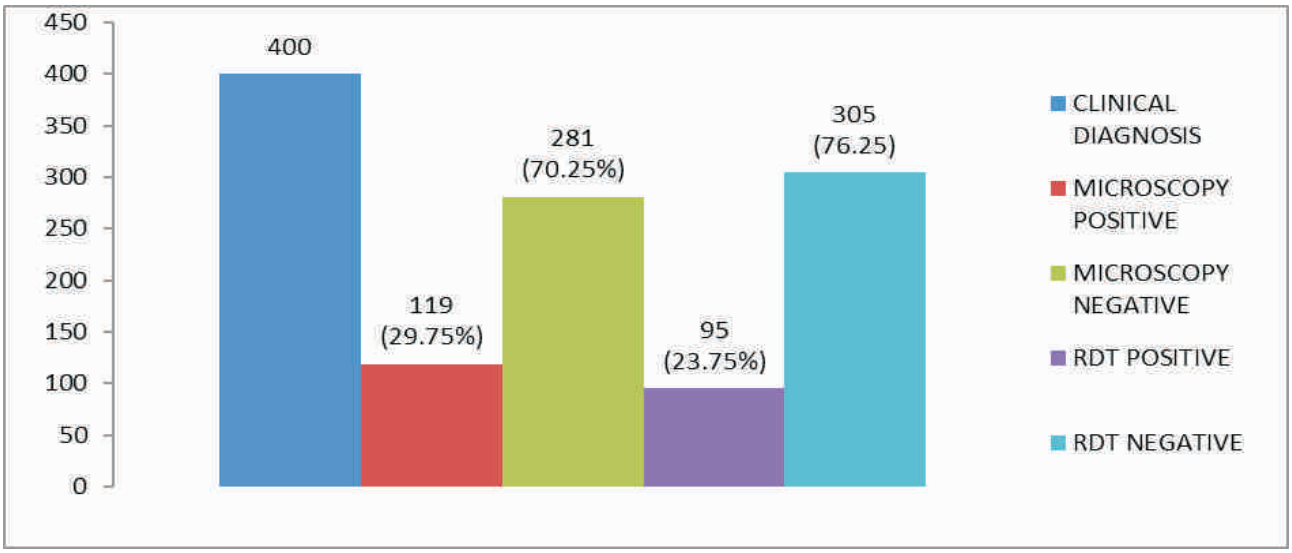

Figure 1: **Clinical diagnosis, RDT and microscopy results summary ($x^2 = 5.11$, $p \approx 0.20$)**

Table 3: Relationship between malaria and anaemia in children (0-5 years) on clinically suspected malarial patients

Location	Total Patients (0-5 Years)	Anaemic, MP Positive	Non-anaemic, MP Positive	Anaemic, MP Negative	Non-anaemic MP Negative	Clinically anaemic with Normal PCV
GHKTN	44	12	7	2	23	0
GHMANI	16	7	O	4	5	2
TUYMCH	48	7	14	7	20	7
TOTAL/%	108	26 (24.1)	21 (19.4)	13 (12.0)	48 (44.4)	9 (0.8)

($X^2 = 0.416$, P= 0.95)

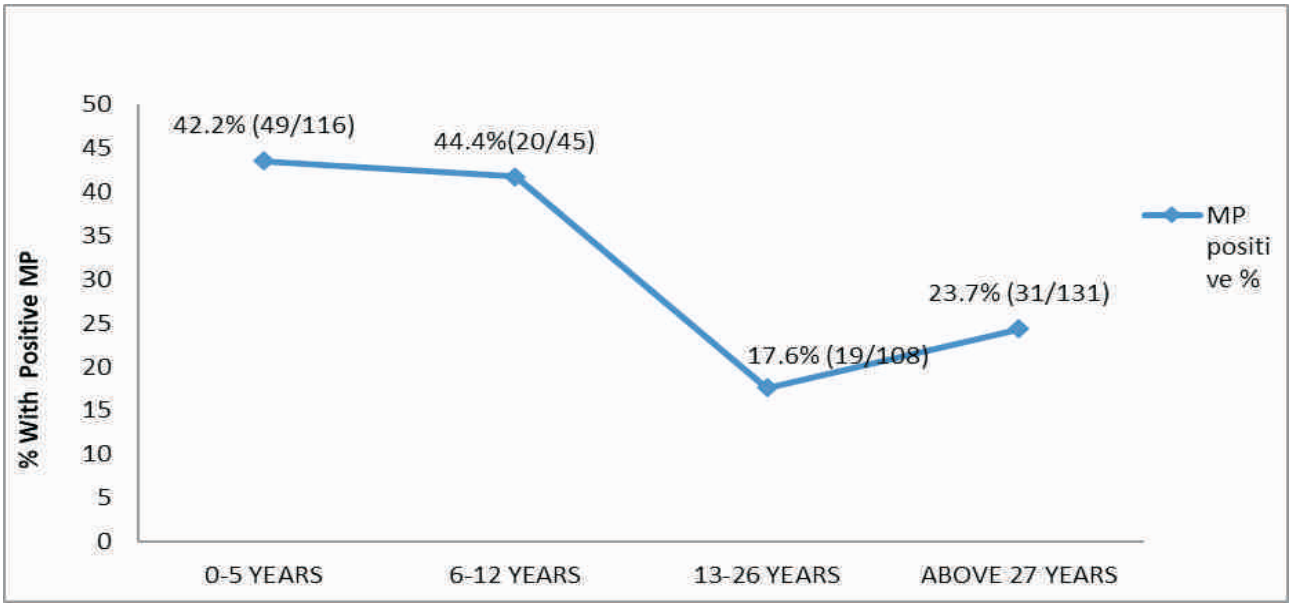

Figure 2: Relationship between malaria and age (r = -0.919)

Discussion

There are basically four major methods used in malaria diagnosis. These are symptomatic (clinical diagnosis), microscopy, antigen test and molecular methods. None of the hospitals in Katsina state employs molecular method in malaria diagnosis. Symptomatic diagnosis is the most common and often the method used alone to diagnose malaria. In other hospitals, symptomatic diagnosis is often the initial one, followed by one of the other methods. However, it should be noted that many other diseases present symptoms very similar to malaria, and diagnosis by symptoms alone can be misleading and even harmful. The first symptoms of malaria (Onset of long periodic fevers, chills, sweats, headaches, muscle pains, nausea and vomiting) are often not specific and the diagnosis is often wrong as these symptoms are also found in other diseases (such as sickle cell crisis, some Bacterial and common viral infections). Likewise, the physical findings are often not specific (elevated temperature, perspiration, tiredness, chills, and body pains are often taken to be symptoms of malaria). Almost all the patients had fever as one of their complaints, but only 29.75% of the patients were found to be infected. This shows that most febrile cases are not due to malaria. This supports the findings of Chansuda *et al.* (2007).

In contrast to the findings of Dicko *et al.* (2005), of which they reported that, malaria is the main cause of fever. The mean PCV of malarial positive patients was lower than mean PCV of MPs negative. This is in support of previous reports (Nicholas *et al*., 2018; Idris *et al.,* 2015) which independently verified that malaria infected patients, are more prone to low PCV. However, the findings indicated that there was no significant difference between the two groups. The research also shows that anaemia alone cannot be used as an index to determine malaria in children.

Malaria prevalence was highest within children using both microscopy and RDTs. The age distribution of prevalence indicates an increase in parasite prevalence from infanthood to older children as the population ages. Statistically however, there was no significant relationship between malaria and age. Our finding is in conformity with the findings in previous reports (Wobo *et al*., 2014; Ike *et al.,* 2008; Mwangi *et al.,* 2005) which found out that children are more vulnerable to malaria infection. Some parents, mostly from rural areas do not bring their children early when they are sick. It was only when the sickness became unbearable that they then rush to hospitals, and that was the more reason for anaemia mostly in children.

Males were generally more prone to malarial infections than females. This agrees with previous reports (Wobo *et al*., 2014; Mendel and White, 1994; Ukpai, 2001). Studies have shown that females have better immunity to malaria and varieties of other parasitic diseases and this was attributed to hormonal and genetic factors (Portilo and Sullivan,1997; Mendel and White, 1994) suggested that genetic factors could play a role by endowing females with immuno-regulatory potentials to cope better with some disease infections. This may invariably be attributed to the fact that males are more exposed to the bites of mosquitoes and other vectors than females, especially when the weather is hot and during farm work. Exception is found during pregnancy and reproductive ages, when females are more prone to malaria attacks due to immune suppression (Wobo *et al*., 2014).

The positivity rate was generally lower than expected. All the 400 patients that participated in this project were attested by clinicians to be sick and their ailment was suspected to be caused by malarial parasites. Out of these clinically suspected patients, only 29.75% turned out to be actually infected with malaria parasites. The findings generally showed that clinical judgements have little utility in malaria diagnosis and there is no single algorithm that can be used as a universal indicator. The markers normally used are vague and imprecise in determining actual patients affected with malaria disease. This postulation is consistent with previous reports (Andrea *et al.,* 2016; Dicko *et al.,* 2005; Mwangi *et al*.,2005).

Eighty per cent of the microscopy-detected infections were *P. falciparum*, while the remaining was either mixed species infections of *P. falciparum*

with *P. malariae* or *P. ovale.* Others are mono-infections with either *P. malariae* or *P. ovale*. Our finding is at variance with a previous report (Bawa *et al.,* 2015) which detected only *P. falciparum* among pregnant women in Katsina metropolis.

Comparison on the proportions of positive test results between RDT and microscopic diagnosis revealed that Microscopy had a higher sensitivity rate, compared to the RDT results. This shows that Microscopy is more sensitive than *Pf* HRP2 RDT kit. It confirmed the findings of Azikiwe *et al.* (2012) and Mukry *et al.* (2017) who reported that microscopy remains the gold standard for malaria diagnosis. However, there was no significant difference statistically between the microscopy and pfHRP2 RDT kit.

Conclusion

The result of this study showed that clinical diagnosis cannot be relied upon for accurate malaria diagnosis in Katsina state. The detection of *P. falciparum* by the RDT was as reliable as microscopy. Parasite-based diagnosis (microscopy and RDT) should be used in all cases of suspected malaria with the possible exclusion of children in high-prevalence areas and certain emergency circumstances. There should be an established malaria microscopy and RDT quality assurance (QA) and quality assessment agencies in all local, state and national levels.

Conflict of interest

The authors have no conflict of interest to declare.

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