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Diagnostic value of rapid test for malaria among febrile neonates in a tertiary hospital in North-East Nigeria: a prospective cross-sectional study

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ABSTRACT

Objective The WHO recommends testing using microscopy or rapid diagnostic test (RDT) before treatment for malaria. However, the use of RDT to diagnose neonatal malaria has not been widely validated with most studies limited to the first week of life. Thus, we conducted this study to determine the utility of RDT in the diagnosis of congenital and acquired malaria in febrile neonates in Nigeria.

Design This prospective cross-sectional descriptive study consecutively recruited 131 febrile neonates at the Special Care Baby Unit (SCBU) of the Federal Teaching Hospital Gombe, Nigeria. All study participants concurrently had RDT (HRP2, LDH) and malaria microscopy. The performance of both methods was then compared.

Result Seventy-eight of 131 neonates tested for malaria by blood smear microscopy demonstrated malaria parasites; a prevalence of 59.5%. Parasite count ranged from 16 to 520/μL and the median parasite count was 81.0/μL with IQR (40.0–134.5). The majority of patients (93.5%) had low-density parasitaemia ($\leq 2+$). All species identified were *Plasmodium falciparum*. None of the 131 neonates tested positive on RDT.

The sensitivity and positive predictive value of RDT for neonatal malaria was zero. Congenital malaria was the most common form of neonatal malaria, accounting for 75.6%, while acquired and transfusion-related malaria were estimated at 12.8% and 11.6%, respectively.

Conclusion The RDT used in this study was not sensitive in the diagnosis of congenital or acquired neonatal malaria; therefore, microscopy remains the preferred method of diagnosis of neonatal malaria.

INTRODUCTION

Neonatal malaria refers to the presence of erythrocytic asexual form of plasmodium in the first 28 days of life. Malaria in a neonate can be congenital (transplacental transmission), blood transfusion-related or acquired malaria infection.¹ It is considered congenital malaria when asexual parasites are detected in the peripheral blood within the first week of life.² Transfusion-related malaria is the presence of malaria parasite in the peripheral blood of a neonate whose blood was previously negative for malaria parasite prior to transfusion, while acquired neonatal malaria occurs within the first 28 days of life due to infective mosquito bite

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Rapid diagnostic tests (RDTs) have been unreliable in diagnosing congenital malaria occurring in the first 7 days of life.

WHAT THIS STUDY ADDS

⇒ RDTs demonstrate poor sensitivity regardless of the age of the neonate and route of acquisition of the parasite within the first 28 days of life.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ RDT may not be the diagnostic test of choice or an alternative to microscopy in the diagnosis of neonatal malaria.

after an incubation period of at least 1 week.^{3–5} Malaria in the newborn was previously thought to be an infrequent cause of clinical illness, however, parasitaemia may be prevalent in endemic areas and contributes to low birth weight, anaemia and stillbirth.^{5–7} Death in newborns with malaria parasitaemia in cases confirmed by microscopy has been reported to be as high as 25%, therefore the need for prompt and accurate diagnosis and treatment of neonatal malaria.⁸

Rapid diagnostic tests (RDTs) for malaria are immunochromatographic assays which detect malaria antigens in the blood along a membrane containing specific antimalarial antibodies.^{9–10} RDTs were developed out of a need to establish the presence of malaria parasitaemia at the point of care even where there are no laboratories or skilled microscopist.¹¹

Some previous studies on neonatal malaria demonstrated malaria parasites in blood smears of neonates presenting with risk factors or features of neonatal sepsis; these studies however utilised small sample sizes and there were no comparison between RDT and microscopy (the Gold standard).^{12–14} Studies on RDT conducted in neonates were mainly for congenital malaria in which those above 1 week of age were excluded.^{15–18} We conducted this study to bridge these gaps.

METHODS

The study was carried out in the Special Care Baby Unit (SCBU) of the Federal Teaching Hospital



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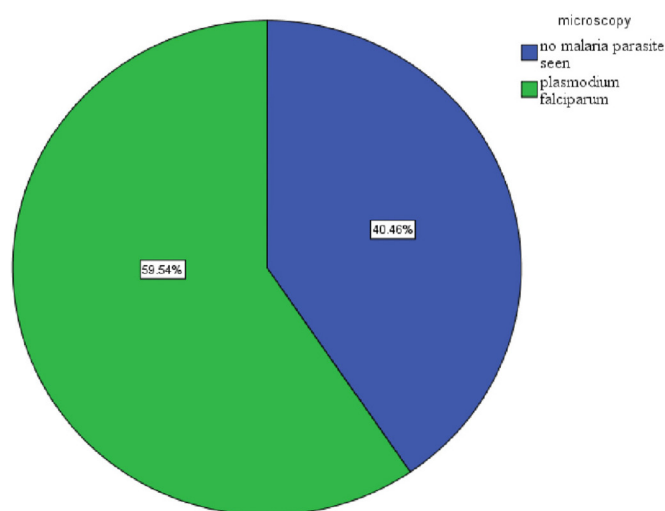
Table 1 Sociodemographic characteristics of study subjects

Variable	Total N=131		
Malaria parasite	Positive	Negative	
Age (days)	78 (59.5%)	53 (49.5%)	
0–7	67 (77.0)	33 (62.3)	100
>7	11 (33.0)	20 (37.7)	31
Gestational age			
Preterm	27 (34.6)	10 (18.9)	37
Term	51 (65.4)	43 (81.1)	94
Place of birth			
FTHG	49 (62.8)	32 (60.4)	81
SSHG	2 (2.6)	4 (7.6)	6
PHC	20 (25.6)	13 (24.5)	33
Private facility	3 (3.9)	0 (0.00)	3
Home	4 (5.1)	4 (7.6)	8
Social class			
Low	20 (25.6)	16 (30.2)	36
Middle	18 (23.1)	20 (37.7)	38
High	40 (51.3)	17 (32.1)	57

FTHG, Federal Teaching Hospital Gombe; PHC, Primary Health Centres; SSHG, State Specialist Hospital Gombe.

Gombe. The unit is divided into inborn section which has a capacity of 15 beds and outborn section with 17 beds. The average admission rate is 60 neonates per month. There is a side laboratory equipped to perform packed cell volume, urinalysis, random blood glucose, rapid test for malaria, Giemsa staining and a microscope for viewing slides for malaria parasite. The personnel in the unit include 3 consultant paediatricians, 5 resident doctors, 3 house officers, 25 nurses and 27 support staff.

The study population comprised febrile neonates (0 to 28) days who presented to SCBU of the Federal Teaching Hospital Gombe or developed fever while on admission in the unit. The study was a prospective cross-sectional descriptive study. The sample size was calculated using Fischer formula for minimum sample size estimation for cross-sectional studies.¹⁹ The prevalence rate of neonatal malaria in sick neonates (8.25%) reported by Orogade *et al*⁶ in the SCBU of a teaching hospital in Northern Nigeria was used²⁰ due to the similarity in facility type, age range of newborns and geographical location. The confidence limit was set at 95%, margin of error was 5% and complementary

**Figure 1** Distribution of malaria parasitaemia results among study subjects.

probability was 0.918. Ten per cent of the calculated value was added to account for incomplete or missing data. The minimum sample size calculated was 128 newborns.

Neonates who met the inclusion criteria were recruited consecutively until the desired sample size was achieved. The definition of fever used in this study was axillary temperature greater than or equal to 37.5°C as stated by WHO.²¹ Neonates who had received antimalarial drugs before presentation were excluded from the study. Afebrile newborns who received blood transfusion during the study period were screened for malaria prior to transfusion using microscopy and those who were negative and subsequently developed fever were recruited into the study. Those who tested positive were classified as having transfusional malaria. Those without a history of transfusion who were positive for malaria within the first week of life were classified as congenital malaria, while those in the second to fourth week of life were classified as acquired malaria.

Sample collection

For each subject recruited, blood samples were collected and labelled using standard technique by WHO within 30 min of subject recruitment.²² The big toe of the left foot was cleaned using cotton wool dipped in alcohol to remove dirt and oil then dried with clean cotton using firm strokes to stimulate blood circulation. A sterile lancet was used in a quick rolling action to puncture the ball of the big toe. Gentle pressure was applied to express the first drop of blood, which was wiped away with dry cotton wool making sure no strands remained. Subsequent drops of blood were collected quickly in a transfer device (capillary tube with a capacity of 5 µL) provided in the RDT kit. SD Bioline Malaria Ag Pf (HRP2/pLDH) lot number 05FDD002A, which tests for *Plasmodium falciparum* histidine-rich protein 2 and lactate dehydrogenase was used in this study. The blood was placed in the appropriate well on the RDT where it was adsorbed by the nitrocellulose paper. Tests were carried out at the SCBU by the researchers according to the manufacturer's instruction. A positive test was indicated by both a test line and a control line, a negative test by only a control line and an invalid test by a test line only on the kit. For further quality assurance, positive and negative control blood samples from the parasitology laboratory (confirmed by microscopy) were used to randomly test two test strips from each pack of RDT, which contained 25 strips and there was 100% concordance between RDT and the gold standard (microscopy).

From the same prick, an additional 2–3 drops of blood for a thick blood smear and a drop of blood for a thin blood smear were collected onto the slides. Thick and thin blood films were Giemsa stained according to WHO standard for malaria parasite staining 2016.²² Parasite density was determined using thick films, and the number of parasites per 200 white blood cells assuming a white blood cell count of $8 \times 10^9/L$ was used to calculate parasite/µL. If no parasite was visualised after viewing 100 high power fields, the blood film was considered negative.²³ The plus system of grading parasite density was used for subjects positive by microscopy (when the parasite was visualised) after discrete values of parasite density were noted.²⁴

Ethical consideration

The research was at no financial cost to the participants and all results were kept confidential. Febrile neonates found to be positive for malaria using microscopy were admitted and treated for malaria. Febrile neonates who did not test positive for malaria

Table 2 Comparison between blood film microscopy and RDT for malaria

Test (RDT)	Parasitaemia (microscopy)	No parasitaemia (microscopy)	
	Positive	Negative	
Positive	TP 0	FP 0	0
Negative	FN 78	TN 53	131
Total percentage	59.5	40.5	100

FN, false negative; FP, false positive; TN, true negative; TP, true positives.

or considered to have co-morbidities were further evaluated and managed accordingly.

RESULTS

A total of 131 neonates aged 0–28 days with fever were studied over a period of 4 months (March to June) 2020. The median age was 4.0 days with IQR of 4. The sociodemographic characteristics of subjects are demonstrated in table 1.

Malaria parasite was visualised in 78 (59.5%) subjects. *P. falciparum* was the only specie identified in malaria-positive subjects. This is shown in figure 1.

Prevalence of malaria using RDT

None of the subjects studied tested positive using RDT. False-negative RDT results were 78 (59.5%). The sensitivity, specificity, positive predictive value and negative predictive values are illustrated in table 2.

Table 3 Grading of malaria parasite density in subjects by plus system

Variable	Frequency n=78	Percentage (%)
+	20	25.6
++	53	67.9
+++	5	6.4
++++	0	0.0

$$\text{Sensitivity} = \text{TP} / (\text{TP} + \text{FN}) = 0 / (0 + 78) = 0$$

$$\text{Specificity} = \text{TN} / (\text{TN} + \text{FP}) = 53 / (53 + 0) = 1$$

$$\text{Positive predictive value} = \text{TP} / (\text{TP} + \text{FP}) = 0 / (0 + 0) = 0$$

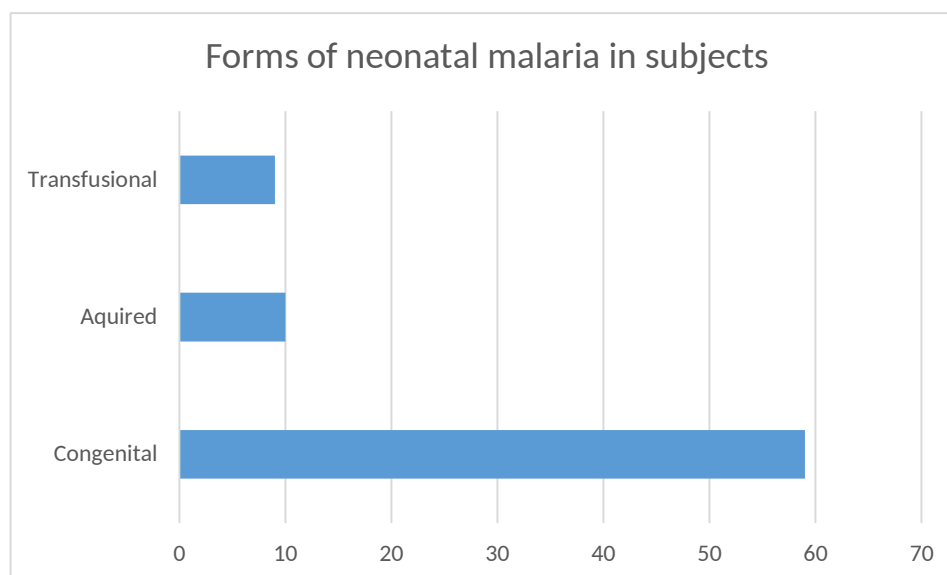
$$\text{Negative predictive value} = \text{TN} / (\text{TN} + \text{FN}) = 53 / (53 + 78) = 0.40$$

The parasite count ranged from 16 to 520 parasites per μL . Median parasite density was 81 with IQR (40–135). Using the plus system, majority of subjects had parasite density of 2+ (67.9%), none had parasite density of 4+. This is summarised in table 3.

Congenital malaria was the most common form seen in this study and accounted for 59 (75.64%) cases. Acquired neonatal malaria accounted for 10 (12.82%) cases of neonatal malaria, while 9 (12.82%) had transfusion-related neonatal malaria. The various forms of neonatal malaria are shown in figure 2.

DISCUSSION

In our study, RDT performed poorly with none of the 78 cases of parasitaemia detected in a sample of 131 newborns with fever. This is similar to findings in studies conducted in West, Central and East Africa^{15 25 26} although only newborns in the first week of life were included in those studies. This could have been due to low parasite densities in neonatal malaria as documented in their studies as well as ours. The performance of RDT improves with higher parasite density.²⁷ Parasite density greater than 100/ μL is more likely to give a positive RDT result^{27 28}; however, in our study more than half of the subjects had parasite density less than 100/ μL . Five subjects with higher parasite densities (+++) still tested negative by RDT. Possible reasons include the presence of haemoglobin F in neonatal blood, which is known to retard parasite growth and may affect sensitivity of RDT.²⁹ In addition, combined deletion of pfHRP2 and pfHRP3 gene and other variations in the HRP2 gene have been reported in the literature and can lead to false-negative RDT results using test strips that detect HRP2 as used in this study^{30 31} and the presence of antibodies (anti-HRP2) in serum of neonates can result in false-negative RDT results.³² Akpiri and Agi¹⁷ reported a better sensitivity using RDT than reported in this study as 50% of neonates positive by microscopy were also positive by RDT. In the study, 26 newborns were screened over a 1-year period and name of the RDT kit (HRP2) used was not documented.

**Figure 2** Forms of neonatal malaria in study subjects.

Different brands have different sensitivities, and the parasite density in that study ranged from 50 to 1020/ μ L.¹⁷

The only malaria specie documented in this study was *P. falciparum*. This is similar to findings by most researchers in Nigeria and Africa,^{17 20 33–36} however, other species (*Plasmodium vivax* and *Plasmodium ovale*) have been reported in India, Indonesia and Italy.^{37–41} This may be due to difference in geographic location as *P. falciparum* is the most common species in Nigeria.⁴² Overall, the parasite density was low as 93.5% of subjects with malaria had one or two plus parasitaemia (+, ++). This is similar to the findings by Ojukwu *et al*³³ in which 93.8% had the same, however, Okechukwu *et al*⁴³ reported a lower rate of 52%. This could be due to different methods of estimating parasite density and reference value for categorisation of parasitaemia.

The prevalence of neonatal malaria by microscopy in this study was 59.5%, which suggests a high burden of malaria parasitaemia among pregnant women and neonates with fever in our setting. This is similar to findings from prospective studies of sick newborns in neonatal unit that included both term and preterm babies.^{20 23} Hyacinth *et al*⁸ documented a prevalence of 58.5% while Opara and West reported 43.7%.³⁴ However, studies that excluded preterm neonates reported lower prevalence (38.9%)³³ despite being conducted in similar settings on sick newborns with retrospective methods reporting even lower rates (24.8% and 8.25%).^{20 44} This disparity may not be surprising as preterms constituted a significant part of the burden of malaria in our study and maternal malaria is a known cause of preterm delivery.

Congenital malaria has consistently accounted for the majority of cases of neonatal malaria with percentages ranging from 61% to 84%^{8 34 36 43} and two studies documented 75% as seen in this study.^{33 45} In studies with all three forms of neonatal malaria, acquired and transfusional malaria seem to occur with similar frequencies (12.8% and 11.5%) in this study which is similar to (13% and 13%) documented by Ibhanebor⁴⁵ in Benin City. The similarity could have been due to characteristics of subjects in both studies (sick neonates with or without history of blood transfusion).

In conclusion, the RDT for *P. falciparum* used in this study was not sensitive as a tool for malaria diagnosis in newborns, and although parasitaemia among febrile newborns was common in north-east Nigeria, parasite densities were generally low.

Limitation

In our study, only one RDT brand was used to compare with microscopy and findings may not be generalisable to all malaria RDTs.

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Contributors Conceptualisation of study was done by YRA, IJ, IO, EIW, OW, MM, MRP and HA. Data collection was done by YRA, MRP, HA and IJ. Analysis and interpretation of data was conducted by YRA, IJ, IO, OW, EIW, MM and MRP. Drafting and reviewing of manuscript was done by YRA, IJ, IO, MRP, OW, MM, EIW and HA. The guarantor is YRA

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by the Hospital Research and Ethics Committee of Federal Teaching Hospital Gombe with reference number NHREC/25/10/2013. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request.

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