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RELEVANCE OF URINE RAPID TEST IN THE DIAGNOSIS OF MALARIA AMONG FEBRILE CHILDREN AGED 1-10 YEARS IN SOUTH EASTERN NIGERIA

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ABSTRACT

Background: Early diagnosis, prompt and effective treatments are the basis for the management of malaria and key to reducing the associated morbidity and mortality. Laboratory diagnostic challenges exist because clinical diagnosis is imprecise and leads to excessive use of antimalarial drugs. Thus there is need to introduce other non-invasive rapid diagnostic tools in order to achieve the goal of universal access to malaria diagnosis.

Aim: The aim of this study was to determine the reliability and usefulness of urine malaria test in the diagnosis of malaria among febrile children aged 1-10 years.

Materials and Methods: A cross-sectional descriptive study in which 212 febrile children aged 1-10years were consecutively recruited. Socio-demographic and clinical data of the subjects were obtained using interviewer-administered questionnaires. Each of the subjects were screened for malaria using blood and urine based malaria test kits respectively. Blood samples from each subjects were also sent to the laboratory for malaria microscopy.

Results: Out of 212 subjects studied, 38 subjects (17.9%) had malaria parasitaemia based on microscopy. The prevalence of malaria based on blood-based malaria rapid diagnostic test and urine malaria test were 21.7% and 9% respectively. Microscopy as the gold standard for malaria diagnosis compared to other diagnostic modalities.

Conclusion: In view of the low sensitivity of urine malaria test in this study, there is need for further improvement in the sensitivity of urine malaria test before it can be used alone as screening/diagnostic test for malaria in our locality.

Key Words: Diagnosis, Laboratory, Urine, Anti-malaria, Parasitaemia

INTRODUCTION

Malaria is a major cause of morbidity and mortality among children in developing countries.[1] Despite the global effort in prevention and control of the disease, progress in reducing the burden of malaria has stalled since 2017.[2] The World Health Organization has issued a directive that there is need for urgent targeted action especially in Nigeria where the number of cases are increasing rather than reducing annually.[2]

Early diagnosis, prompt and effective treatment are the basis for the management of malaria and key to reducing the morbidity and mortality associated with the disease.[3] Parasite-based diagnosis of malaria prior to treatment with antimalarial is fundamental to this goal because clinical diagnosis is imprecise and leads to over diagnosis of malaria with resultant wastage of antimalarial medicine and development of drug resistance.[4,5] Accurate and rapid diagnosis of malaria in endemic areas is particularly important in children and non-immune populations in whom falciparum malaria can be rapidly fatal.[1,3]

Blood film microscopy is the gold standard for the diagnosis of malaria, which depends mainly on the experience of the Microscopist, quality of the slides, stain, microscope, time spent in examination of the slides and the fact that it is not available in resource-limited settings.[6] The World Health

Organization have recommended the use of blood-based rapid diagnostic test as an acceptable, accurate and rapid method for the parasitological diagnosis of malaria.[7] However its use requires collection of blood by lancet/needle prick which carries some risk of injury and disease transmission.[8] Furthermore, in Africa, cultural and religious beliefs can also be an obstacle to the use of blood based malaria rapid diagnostic test (BMRDT).[9] Therefore, there is need to continue the search for an alternative or complementary malaria rapid diagnostic test that will employ other non/less invasive specimen apart from blood.

Histidine rich protein 2 (HRP-2) is a water soluble protein and could potentially be detected in urine since urine is an Ultra-filtrate of plasma.[10] Thus urine could be used as an alternative body fluid for the detection of plasmodium falciparum malaria. Histidine rich protein 2 (HRP-2), a protein produced by P.falciparium and has been proven from previous studies that it can be detected in urine of individuals infected with P.falciparium malaria[11]. The protein is believed to be excreted ito the blood stream and then removed via ultra filtrtion in the kidney into the urine[12]. It (HRP-2) has been explored as a biomarker for malaria diagnosis due to its high sensitivity and specificity in detecting P.falciparium presence and infection[13] Therefore, its presence in urine marks it as a promising target for non-invsive diagnostic tool. It is important to note that the detection of HRP-2 in urine has significant implications for malaria diagnosis, particularly in resource-poor settings like Nigeria. Non-invasive urine based test could favour a non-invasive and rapid diagnostic tool, improving access to malaria diagnosis and treatment[14]. The aim of this study was to determine the reliability and usefulness of urine malaria rapid diagnostic test (UMT) in the diagnosis of malaria among febrile children aged 1-10 years.

MATERIALS AND METHOD

Study Area and Design

This was a cross-sectional study carried out between 3rd of May to 5th of August 2019 at Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, South-East Nigeria. The hospital is one of the two tertiary health institutions in Anambra State. Nnewi is a commercial city located in Nnewi North local Government Area. It has a population of 391,227 based on 2006 census estimate[15]. The inhabitants are predominantly Igbo-speaking and are mainly traders and civil servants. Nnewi is located on latitude 6^0 01' N of the equator and longitude 6^0 55' E of the Greenwich Meridian.[16] It has a mean daily temperature of 30.4^0C and mean annual rainfall of about 2000cm[16]. Nnewi falls within the tropical rainforest region of Nigeria with 2 main seasons; the rainy season spanning from April to October, and the dry season spanning from November to March[16]. The hospital, NAUTH, amongst other services maintains a Children Out-Patient Clinic (CHOP) that is open from Monday to Friday every week and a Children Emergency Room (CHER) that is open 24 hours daily. Even though NAUTH is supposed to be a referral center, the hospital functions as primary, secondary and tertiary health-care facility as many patients from the community present here for the first time without any referral.

Study Population

The study population consisted of febrile children aged 1-10 years who presented to the CHOP and CHER of the hospital. Inclusion criteria were axilliary temperature ≥ 37.5^0c and history of fever in the preceding 48hours. Children who had received full course of Artemisinin Combination Therapy (ACT) in the index illness or on malaria prophylaxis prior to the onset of the current illness were excluded.

Subjects Recruitment

Written informed consent was obtained from the subjects' caregivers/parents while assent was obtained from subjects aged 7 years and above. The subjects who met the inclusion criteria were consecutively recruited into the study until the sample size was reached. The information obtained from the subjects included bio-data, such as age, sex, parental occupation and highest educational level of the parents. Socioeconomic class of the subjects were grouped into low, middle and high class.[17]

Laboratory Procedures

The preparation and reading of the thick and thin blood film for malaria microscopy was done with the assistance of two Laboratory Scientists trained and certified in malaria microscopy by the World Health Organization. The Laboratory Scientists that assisted in the study were blinded to the history and examination findings of the subjects. While maintaining aseptic and universal safety precautions, two millilitre of blood were collected from each child into an ethylene diamine tetra acetic acid (EDTA) bottle. Each EDTA bottle was assigned a code number and the blood were subjected to test within 24 hours of collection. Thick and thin blood films were prepared on a single slide for each of the sample: 6µl of blood for the thick film and 2µl for the thin film. Three percent working Giemsa stain was prepared with stock of Giemsa staining solution and working Giemsa buffer. The thick and thin blood films were stained for 45 minutes with working Giemsa stain after fixing the thin film with absolute methanol.[18] Once the staining was over, the slides were air dried on a rack. Each slide was examined microscopically using 100x objectives (oil immersion). At least 100 oil immersion fields were examined before reporting the slide as negative.[18] Thick films were used to determine the parasite density while the thin films were used to identify the parasite species. The blood film was said to be positive when a concordant result was produced by the two Microscopists. The parasite density per micro-liters of blood was estimated from the thick film by counting the number of asexual parasites per 200 white blood cells expressed as parasites /µl (micro-litre) assuming a total WBC count of 8000/µl.[19]

$$\frac{\text{number of parasites x total leukocyte count}}{200}$$

Blood and urine samples from each of the subjects were screened for malaria using BMRDT and UMT kits. It is very vital to note here that the urine samples were collected as the patients present in Clinic, that is, anytime the patients presents. The procedure was carried out by the researchers according to the manufacturer's guideline. The rapid diagnostic tests and microscopy were performed independent of each other. The rapid diagnostic test kits (BMRDT and UMT, kits) were supplied by the manufacturers. BMRDT kits were produced by standard diagnostic INC based in Korea LOT NO: 05CDDO26A while the UMT kits were produced by Fyodor company based in Maryland USA LOT NO: F8001.

Ethical Clearance

Ethical clearance was obtained from the health research and ethics Committee of NAUTH Nnewi. Informed consent was

obtained from each caregiver and assent from children who were 7 years and above after educating them on the need for the study.

Determination Of Sensitivity And Specificity:

The sensitivity and specificity was calculated using the formula:

$$\text{Sensitivity}(\%) = \frac{TP}{TP + FN} \quad X\ 100,$$

where TP =True positive, FN= False Negative

$$\text{Specificity}\ \% = \frac{TN}{TN + FP} \quad X\ 100$$

Where TN= True Negative, FP= False Positive

Determination of positive and Negative Predictive Value:

$$\text{PPV} = \frac{TP}{TP + FP} \quad X\ 100$$

$$\text{NPV} = \frac{TN}{TN + FN} \quad X\ 100$$

TP= True Positive
FP= False Positive
TN= True Negative
FN = False Negative
PPV = Positive predictive value
NPV = Negative predictive value

Data Analysis

The independent and dependent variables were categorized accordingly and association was compared using contingency tables such as Chi-square (x^2) or Fischer's exact analysis where appropriate. The *p-value* was considered statistically significant at ≤ 0.05. The dependent variables: the parasite densities, UMT and BMRDT results were expressed in proportions (percentages), categorized for determination of sensitivity, specificity and predictive values (positive and negative).

RESULTS

A total of 212 subjects were recruited and successfully studied. There were 134 (63.2%) males and 78 (36.8%) females, giving a male: female ratio of 1.7:1. Their age ranged from 1 to 10 years, with a median age of 4 years. Children less than five years were the highest in the study population consisting 58% of enrolled subjects while children from the age of 5 to 10 years were the least (42%) (see Table 1). Axillary temperature ranged from 35.3^{0}C to 40.3^{0}C with a mean of 37.5±1.1^{0}C. The mean number of days the subjects had fever before presentation was 3.5 ±2.4days with a range of 6 hours to 14 days. Thirty-eight subjects (17.9%) were positive by microscopy, 46 (21.7%) were positive by BMRD while 19(9%) were positive by UMT (Figure 1).

Seventeen subjects had corresponding positive microscopy and UMT results (true positive tests) whereas 172 subject were negative by both diagnostic method (true negative tests) (see Table 2). Also, thirty-seven subjects had a corresponding positive microscopy and BMRDT results (true positive tests) and 165 subjects were negative by both diagnostic test methods (true negative tests) (see Table 3).

Using microscopy as the gold standard, the sensitivity and specificity of UMT was 44.7% and 98.9% respectively while the positive and negative predictive values were 89.5% and 89.1% respectively. Also, the sensitivity and specificity of BMRD was

97.4% and 94.8% respectively. The positive and negative predictive values of BMRD was 80.4% and 99.4% respectively (Table 4). The sensitivity of BMRDT increased as parasite density increases while that of UMT increase and decrease as parasite density increases (Table 5).

Table 1: Background characteristics of the subjects

Variable	Frequency	Percentage(%)
Age (years)		
< 5	122	58%
≥ 5	90	42%
Gender		
Male	134	63.2
Female	78	36.8
Socioeconomic class		
Upper	34	16.0
Middle	114	53.8
Lower	64	30.2
Temperature at presentation		
≥ 37.5ºC	104	49.1
< 37.5ºC	108	50.9

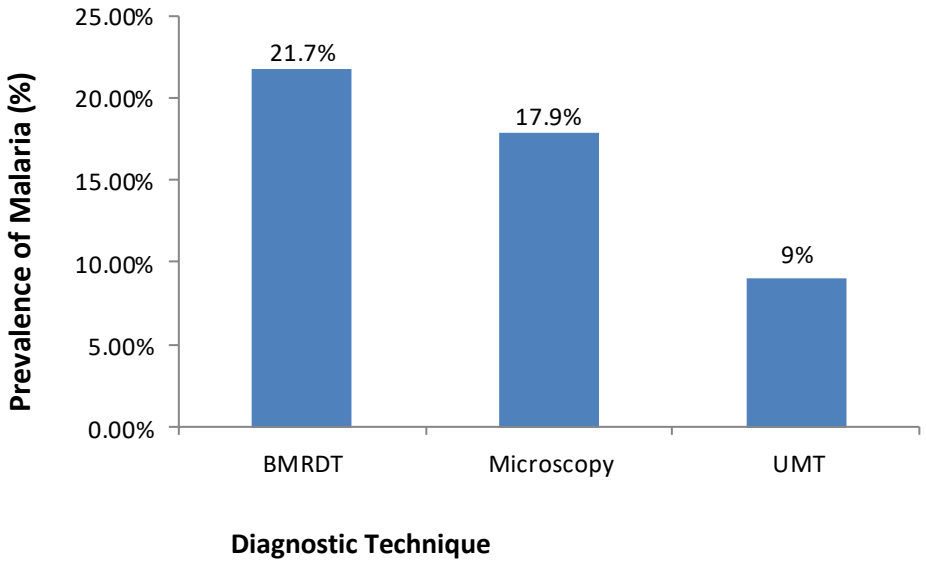

Figure 1:Bar chart showing the prevalence of malaria parasitaemia based on the different diagnostic techniques.

Table 2: Comparison of result of UMT and microscopy

	Microscopy.		
UMT	**Positive**	**Negative**	**Total**
Positive	TP = 17	FP = 2	19
Negative	FN = 21	TN = 172	193
Total	38	174	212

TP = True Positives, TN = True Negatives, FN = False Negatives, FP = False Positives.

Table 3: Comparison of result of BMRDT and microscopy

BMRDT	**Microscopy**		
RDT	**Positive**	**Negative**	**Total**
Positive	TP = 37	FP = 9	46
Negative	FN = 1	TN = 165	166
Total	38	174	212

TP = True Positives, TN = True Negatives, FN = False Negatives, FP = False Positives.

Table 4: Performance evaluation of BMRDT and UMT using microscopy as gold standard

Performance characteristics	UMT	BMRDT
Sensitivity	44.7%	97.4%
Specificity	98.9%	94.8%
PPV	89.5%	80.4%
NPV	89.1%	99.4%
False positive rate	1.1%	5.2%
False negative rate	55.3%	2.6%

PPV= Positive predictive value, NPV= Negative predictive value.

Table 5: Variation of sensitivity of the rapid diagnostic test by parasite density

Parasite density/µl	No. positive by microscopy(%)	No. positive by BMRDT(%)	No. positive by UMT(%)	Sensitivity(%) UMT	BMRDT
(<50/µl)	0	0	0	0	0
(50 -< 500/µl)	3(7.9%)	2(4.3%)	1(2.6%)	33.3%	66.7%
(500 -<5,000/µl)	12(31.6%)	12(31.6%)	7(18.4%)	58.3%	100%
(5000-<50,000/µl)	12(31.6%)	12(31.6%)	1(2.6%)	8.3%	100%
(≥ 50,000/µl)	11(28.9%)	11(28.9%)	8(21.0%)	72.7%	100%

DISCUSSION

The sensitivity of UMT established in this study was quite low (44.7%) while the specificity was very high (98.9%). The sensitivity of UMT observed in this study was below the WHO recommendation for an appropriate malaria rapid diagnostic test while the specificity was consistent with the WHO recommendation[20]. The implication is that at a sensitivity of 44.7% the kit is capable of detecting 44 out of 100 children with malaria. This implies that it will miss more than 55% of the children who have malaria. The sensitivity of UMT observed in this study was comparable to what has been reported in other studies elsewhere[21-23]. However, it was lower than what has been reported in other studies conducted in Nigeria[10,24,25]. Egbuche et al.[26] observed a higher sensitivity of 76.9% in a study conducted in Awka North Local Government Area of Anambra State. Although both children and adults were included in the study. The variations in the sensitivity of UMT in different settings may be due to differences in the methodology or parasite density in the area. The positive and negative predictive value of UMT observed in this study was high and is comparable to what has been reported in other studies[25, 27, 28].

A high sensitivity (97.4%) and specificity (94.8%) of BMRDT was observed in this study. The performance of BMRDT in this study is in line with the WHO recommendation for an appropriate malaria rapid diagnostic test. The sensitivity of 97.4% implies that the test is capable of detecting 97 out of 100 children that have malaria. This means that it will miss less than 3% of children who have malaria. The sensitivity and specificity of BMRDT in this study was comparable to what was reported in other studies, done in Enugu and Sokoto[29,30]. However, the sensitivity was higher than what has been reported in Zamfara State and Delta State[31,32]. The high sensitivity of BMRDT documented in our study may be due to the high parasite density observed.

CONCLUSION AND RECOMMENDATION

The sensitivity of urine malaria test observed in this study was below the standard set by the WHO while the performance of blood based malaria rapid diagnostic test was consistent with the WHO standard. A screening test for a devastating disease like malaria is expected to have high sensitivity. Therefore urine malaria test kits should not be used alone as a screening test for malaria in our locality.

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Conflict Of Interest Disclosure

All the Authors declared that there was no conflict of interest