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Usefulness of rapid diagnostic test in the diagnosis of asymptomatic malaria in HIV infected children on cotrimoxazole prophylaxis in Benin City, Nigeria

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Background: Rapid diagnostic test (mRDT) is a useful tool in demonstrating parasitologically proven malaria. Its efficacy is however hampered when parasite density is low. Prophylactic use of cotrimoxazoleas in cases of HIV infected children can cause reduction in parasite count. It is doubtful if mRDT will retain its diagnostic usefulness among such individuals.

Objectives: The study sought to evaluate the diagnostic value of mRDT in HIV infected children on cotrimoxazole prophylaxis in Benin City.

Methods: In the prospective, cross sectional and descriptive study, we assessed malaria parasitaemia using standard methods in microscopy and parasite density and malaria antigenaemia using Care Start Pf (monoclonal antibodies specific to histidine rich protein – 2 antigen) in 221 each of HIV infected subjects on cotrimoxazole managed in a specialist clinic and HIV negative controls all seen at the University of Benin Teaching Hospital between April and June 2016.

Results: Malaria antigenaemia rate MAr (20.8%) was lower than malaria parasitaemia rate MPr (24.4%) in subjects. MAr (20.8) and MPr (24.4%) in subjects were higher than MAr (18.10%) and MPr (17.7%) in controls. Mean (SEM) parasite count in subjects of was low (50.88 ± 2.24 per µl). Using microscopy as gold standard the sensitivity, specificity, PPV and NPV of mRDT in subjects were 77.8%, 97.6%, 91.3% and 93.1%. Corresponding values in controls were 100.0%, 99.5%, 97.5% and 100.0%.Youden indices for subjects and controls were 0.75 and 0.99.

Conclusions/Recommendations: Sensitivity of mRDT in HIV infected children on cotrimoxazole prophylaxis for opportunistic infections (OI) is reduced. However the indices of specificity, PPV and NPV are high enough to retain its value in the evaluation of HIV infected children for asymptomatic malaria and perhaps the clinical disease.

Keywords: mRDT, Utility, HIV-infected Children, Cotrimoxazole-prophylaxis, Benin City

Introduction

Malaria remains one of the leading causes of morbidity and mortality in children in sub-Saharan Africa.[1] Progress in malaria case management in recent past have resulted from improved case definition through enhanced diagnosis and use of combination therapy in place of the presumptive diagnosis which was popular.[2] Mainstay of diagnosis of malaria is microscopy but it is operator, equipment and light dependent.[3] Sensitivity is also dependent on expertise of the microscopist. These inherent weaknesses can be overcome with the use of malaria rapid diagnostic test (mRDT) based on antigen demonstration using preformed antibodies.[4]Compared to microscopy mRDT in some studies showed95 - 100% sensitivity in the detection of malaria.[5,6]

However sensitivity of mRDT varies with parasite density.[3] Sensitivity is low when counts are low. Falade and co-workers in 2013 in Ibadan evaluated an mRDT (Paracheck-Pf) in the rapid diagnosis of malaria among HIV positive adults (some of whom were on HAART) suspected to have malaria.[3]They found comparable rates for malaria parasitaemia and antigenaemia. They also observed improvements in sensitivity and specificity with higher parasite counts. The authors concluded by acknowledging that the RDT is useful as a diagnostic tool at high parasite densities (>200/microliter). The author however, made no provisions for controls.

HIV/AIDS is also rampant in sub-Saharan Africa.[7-9]The two diseases interact and reinforce each other in terms of

morbidity and mortality. Controlling one can cause marked improvement in the outcome of the other.[9] However most individuals with HIV/AIDS are commonly placed on cotrimoxazole(a fixed–dose combination of sulfamethoxazole and trimethoprim): a drug with an acknowledged anti -malaria effect.[10-12] Patients on cotrimoxazole have been found to have low parasite density[10] implying perhaps that the sensitivity of mRDT in such patients would be compromised. How useful mRDT would be in such patients need to be explored. The study therefore aimed at determining the sensitivity, specificity, positive predictive value and negative predictive value of mRDT as against malaria microscopy in the diagnosis of asymptomatic malaria in HIV/AIDS infected and un-infected children (as controls).

Subjects and methods

Study design is a prospective, cross-sectional one.
Study location:

University of Benin Teaching Hospital (UBTH) was the study location. Participants were recruited from the United States Presidential Emergency Plan for AIDS Relief (PEPFAR) Paediatric Clinic which opens twice a week. The average patient attendance per week is about 48. Of about the 24 patients seen per day, about 8 are new ones. Controls were gotten from the Family Practice Clinic (FPC) which opens every day of the week including weekends and the Consultant Out-Patient Clinic (COPC) of the Hospital. The weekly patient attendance in FPC is about 1100 out of which about 10% are children. The COPC has an average weekly attendance of about 650 patients. UBTH is located in Benin City, a cosmopolitan City which also serves as the Capitalof Edo State. The PEPFAR clinic sub serves the people of Edo and neighbouring States. The local weather in the catchment area is supportive of the propagation of the vector responsible for malaria transmission (mean annual rainfall of 2000 mm, high humidity (74.9%) and mean annual temperature of 26.1^{O}C) and the area is holo -endemic for malaria.

Ethical approval

The Ethics and Research Committee of the University of Benin Teaching Hospital (UBTH) granted approval for the study. Following appropriate counselling, written informed consent were obtained from parent(s)/guardian (s) of subjects and controls (aged less than 10 years) and assent obtained from study population, (if 10 years and above , as specifically adopted for the study as persons of younger ages in the locale are unlikely to appreciate the implication of assenting to a study).

Study population:
Inclusion criteria for subjects and controls.

Subjects were HIV-1 positive children (1-17 years of age) attending the Paediatric HIV clinic in UBTH, who assented to/or whose parents or guardian consented to their participation in the study. Controls on the other hand, were HIV negative children without malaria (aged 1-17 years, age and sex matched) seen at the FPC and COPD in UBTH for non-malaria and HIV related medical conditions (follow up for such illnesses as lower respiratory tract, diarrhoeal diseases and chronic neurologic diseases) who assented to/or whose parents or guardian consented to their participation in the study.

Exclusion criteria for subjects and controls

An otherwise eligible subject or control was excluded if he/she had history of treatment for malaria or features of malaria in the preceding two weeks, was on corticosteroids or had sickle cell anaemia. Also excluded were those who had other chronic diseases/infections, overt malnutrition and those who declined assent to/or whose parents or guardian refused consent to their participating in the study.

Sampling method

Subjects were recruited consecutively until the desired sample size was met (minimum of 165 as determined using the formula for ascertaining point prevalence of a characteristic of interest with known prevalence and population in the defined locale of less than 10,000.[13]Individuals fit for recruitment as control were matched for sex and age as applicable to known subjects and enrolled consecutively.

Pre and post-test counselling

Apparently healthy controls of unknown HIV status had pre and post-test counselling conducted for the parents/ guardians or control if he/she was of age. (Few prospective controls who tested positive for HIV-1 were excluded and subsequently referred to the specialist clinic).

Evaluation

Relevant information on socio demographics, clinical features, drug treatment, anthropometry from each participant, disease clinical stage were entered into a standard proforma. Family socio-economic status (SES) was determined from parents' education and occupation.[14]

Laboratory studies

Two and half millilitres of venous blood was withdrawn from each child and dispensed (2 millilitres into a vacutainer and 0.5 millilitres into another sample bottle with EDTA and mixed thoroughly). From the latter, malaria parasitaemia using microscopy was done according to standard methods.[15,16] Each sample earmarked for malaria parasitaemia was analysed using thin and thick films for asexual forms of the parasite. The degree of parasitaemia was determined for each positive smear. A WHO certified microscopist at the Research laboratory in the Department of Child Health carried out the procedures. Parasite counts were calculated from thick blood smear by relating asexual parasite counts to 200 leuko-

cytes (assuming a count of 8000/microliter) using the method described by McKenzie *et al*.[17]

Determination of malaria antigenaemia

Antigenaemia was determined using the Rapid test for the Detection of Malaria HRP-2 (histidine-rich protein 2) in Human Blood (*CareStart*^{TM} Malaria HRP-2 (Pf) made by Access Bio, Inc. 65 Clyde Road, Somerset, New Jersey 08873,USA and specific for *P falciparum*. In carrying out the test, a drop of blood is placed in a well at one end of the plate and two drops of buffer applied to its well. As the patient's blood containing HRP-2 antigens migrates up the test strip, they react with the monoclonal antibodies already impregnated in the strip. The complexes formed react with another set of antibodies coupled with colloidal gold, which show off as a positive band on the plate. The test is considered positive when colour (irrespective of intensity) bands appear in the test and control apertures, negative if only control band is present and invalid if only the test band is revealed.

HIV screening for controls

HIV screening for controls with unknown HIV status were determined using the Alere Determine TM HIV-1/2 kits (Abbot, Japan).

Follow up

Participants with positive malaria parasitaemia/ antigenaemia were notified, followed up for development of symptoms of clinical malaria and where they did, such persons had artemisinin based combination therapy.

Data/statistical analysis

Data obtained were entered into SPSS statistical software program IBM SPSS version-20. Same software was used for data analyses. Categorical variables were expressed in proportions or percentages. Chi-square test was employed to test for non-associations between proportion(s) in subjects and controls while Student t test was used in comparing means. True positives, true negatives, false positives and false negatives were determined with malaria microscopy serving as the gold standard against which malaria antigenaemia were tested. Sensitivity, specificity, positive and negative predictive values were calculated using standard formulae. Also calculated were the Youden's indices for subjects and controls. The level of significance was set at $p<0.05$ and confidence level at 95% confidence interval

Results

Two hundred and twenty one each of subjects and controls participated in the study. The age and gender distribution of subjects and controls are as contained in table 1. Each of subjects and controls had 121 (54.0%) males and 100 (45.2%) females. The modal age bracket was 9-12 years (74 or 53.5%) (For subjects and controls) while the least represented group was the 1-4 years bracket.

Prevalence of malaria parasitaemia in subjects and controls.

Of the 221 subjects 54 (24.4%) had malaria parasitaemia (MP)(all *P. falciparum*). MP was significantly more in males (28.9%) compared to females (19.0%) (^{2} = 1.40; p= 0.236).Twenty three (59.0%) of the 39 controls with MP were males (19.0%) while 16 or 16.0% were females. More subjects in the age bracket >13 years and 9-13 years among the controls had MP. Parasitaemia was least prevalent in the 1-4 years age group (Subjects and controls). In both groups prevalence of MP was independent of age. (Table 1.)

Table 1: Prevalence of malaria parasitaemia by age and gender among subjects and controls

	Subjects			Controls		
Age in yrs	Male (%)	Female(%)	Total (%)	Male (%)	Female (%)	Total (%)
1-4 (n=30)	8 (22.9)	5 (26.3)	13 (24.1)	4 (17.4)	3 (18.8)	7 (31.7)
5-8 (n=48)	9 (25.7)	5 (26.3)	14 (25.9)	6 (26.1)	4 (25.0)	10 (25.6)
9-13(n=74)	5 (14.3)	4 (21.4)	9 (16.7)	5 (22.7)	7 (43.8)	12 (30.8)
> 13(n=69)	13 (37.1)	5 (26.3)	18 (33.3)	8 (34.8)	2 (12.5)	10 (25.6)
Total	35 (28.9)	19 (19.0)	54 (24.4)	23 (19.0)	16 (16.0)	39 (17.6)

χ^2= 0.83, df = 3; p=0.89 (subjects) χ^2=3.33, df = 3; p=0.34 (controls)

Prevalence of antigenaemia in subjects and controls

Forty six(20.8%) of the 221 subjects had malaria antigenaemia. Antigenaemia was commoner in subjects aged 14-17 years and in 9-13 years age group among controls. More male subjects, 28/121 or 23.1% than female subjects (18/100 or 18.0%) had malaria antigenaemia. Twenty three (19.0%) of 121 male controls in comparison to 17 (17.0%) female controls had antigenaemia. No statistically significant relationships existed between age and prevalence of malaria antigenaemia in both groups.

Malaria parasite counts in subjects and controls

Mean (SEM) parasite count in subjects of 50.88 $\pm$ 2.24 per µl was significantly higher than 21.60$\pm$1.36 per µl obtained in controls (t = 5.35; p=0.0001).

Performance of mRDT in the diagnosis of asymptomatic malaria in subjects and controls.

Table 3 show the performances of the mRDT in comparison with microscopy in the diagnosis of malaria. For mRDT the sensitivity, specificity, positive predictive

and negative predictive values were 77.8%, 97.6%, 91.3% and 93.1% for mRDT in the detection of asymptomatic malaria parasitaemia among subjects. Corresponding figures in controls were 100.0%, 99.5%, 97.5% and 100.0%. Sensitivity of mRDT was low in subjects compared to controls while specificity was comparable in the two groups. Positive and negative predictive values of mRDT in the detection of asymptomatic malaria were slightly higher in controls compared to subjects. The performance of mRDT compared to microscopy in the diagnosis of asymptomatic malaria in subjects was 0.75 or 75% in subjects whereas it was 0.99 or 99.0% in controls.

Table 2: Prevalence of antigenaemia by age and gender in subject and controls

	Subjects			Controls		
Age in yrs	Male (%)	Female (%)	Total (%)	Male (%)	Female (%)	Total (%)
1-4	5 (17.9)	4 (22.2)	9 (19.6)	4 (17.4)	3 (17.6)	7(17.5)
5-8	7 (25.0)	5 (27.8)	12 (26.1)	6 (26.1)	4 (23.5)	10(25.0)
9-13	6 (21.4)	4 (22.2)	10 (21.7)	5 (21.7)	8 (47.1)	13(32.5)
> 13	10 (35.7)	5 (27.8)	15 (32.6)	8 (34.8)	2 (11.8)	10(25.0)
Total	28 (23.1)	18 (18.0)	46 (20.8)	23 (19.0)	17 (17.0)	40 (18.1)

χ^2= 0.35, df = 3; p=0.95 (subjects) χ^2=4.03, df = 3; p=0.26 (controls)

Table 3: Performances of rapid diagnostic test in asymptomatic malaria in subjects and controls

	Parasitaemia in Subjects		Parasitaemia in Controls	
mRDT	Positive	Negative	Positive	Negative
Positive	42[a]	4[b]	39[a]	1[b]
Negative	12[c]	163[d]	0[c]	181[d]
	a+c =54	b+d=167	a+c=39	b+d=182
Sensitivity	= a⁄a+c 77.8%		Sensitivity	= a⁄a+c 100.0%
Specificity	= d⁄b+d = 97.6%		Specificity	= b⁄b+d = 99.5%
PPV	= a⁄a+b=91.3%		PPV	= a⁄a+b=97.5%
NPV	= d⁄c+d=93.1%		NPV	= d⁄c+d=100.0%
Youden's index (sensitivity + Specificity -1)	= 0.75		0.99	

Discussion

Performance of mRDT in the diagnosis of asymptomatic malaria.

Against microscopy as gold standard mRDT performances in this study revealed sensitivity of 77.8% and a specificity of 97.6% in the detection of antigens as evidence of malaria. The sensitivity fell short of expectation for a reliable test instrument as recommended by WHO which should be at least 95.0%. However the specificity was high enough to justify its use in practice. Falade *et al* in 2013 evaluated mRDT in the diagnosis of malaria among HIV positive adults in Ibadan, Nigeria.[3] They recorded a sensitivity of 55.0% and specificity of 89%. Sani *et al* in Sokoto, Nigeria studied the performance of mRDT in the diagnosis of malaria in children aged 6months-12years.[18] They obtained a sensitivity of 90.2% and specificity of 95.4%. Both sets of authors[3,18] observed that the sensitivity and specificity improved with increasing parasite count. Whereas the former authors[3] used Paracheck-Pf, the latter[18] utilized Malaria Pf rapid Device, both specific for *Plasmodium falciparum.* The variations in sensitivity and specificity may be attributed to the type of mRDT used as performances vary with brand, nature of subjects and handling of the mRDT kits. The values obtained in this study are comparable to those recorded by Falade *et al*[3]at parasite count <200/µl but lower than values from the study done in Sokoto.[18] Sani *et al*[18]worked on febrile subjects who should have higher parasite counts compared to afebrile subjects. This could explain the improved performance in that study.

In the current study the positive predictive value (PPV) and negative predictive value (NPV)were 91.3% and 93.1%.Falade *et al*[3] in 2013 recorded PPV and NPV of 55.4% and 98.7% respectively. Corresponding values from the Sokoto study were 93.0% and 93.4%.[17] In the two studies[3,18]and the index one, NPV were comparable but the PPV in the study by Falade *et al*[3] was much lower. This presumably had to do with very low parasite counts in some subjects that were undetectable using mRDT.

The sensitivity of 77.8% obtained in this study is however low compared to the corresponding value obtained in the Sokoto study.[18] The reduced sensitivity may be related to few cases that had very low parasite counts, picked by microscopy but missed by mRDT as it is established that mRDT performance is enhanced by higher parasite count.[3,18] The high PPV and NPV in the extant study would rather strengthen the recommendations for use of mRDT in this cohort of patients. The implications of the findings are that in over 90.0% of cases a positive or negative result on the mRDT panel could implicate or exclude to over 90.0% certainty the chance that the disease of interest is present or absent. Granted the current WHO recommendation for evidence - based management of malaria coupled with the need to use ACT based combination for cost effective interventions in malaria the place of mRDT cannot be over emphasized.[19]

A test of the usefulness of a new investigative instrument measured against a known standard can be evaluated using the Youden's index.[20] By the recommendation of this tool the closer to unity the Youden's index is the more sensitive the test is in determining the presence of the disease of interest. In the present case the Youden's index was 0.75, which in essence justifies the use of the mRDT in this set of cohort. In the study the concordance between microscopy and mRDT was

77.8% whereas the discordance rate was only 22.2% implying that in at least four out of every five cases, the new test instrument should perform satisfactorily.

Performance of mRDT in the detection of malaria in controls

The sensitivity and specificity of mRDT compared to microscopy in the detection of evidence of malaria in controls were 100.0% and 99.5% respectively. These performances are in agreement with most values obtained in previous studies carried out in related subjects.[3,21] The positive and negative predictive indices of 97.5% and 100.0% are also in conformity with results obtained in previous endeavors[3,20]The parameters obtained in controls were higher than those gotten in subjects. This is further buttressed by the concordance rate of 100.0% between mRDT and microscopy in these subjects. In the same vain the Youden's index was closer to unity than that obtained with subjects.

The essence of these findings is that mRDT remains useful in the diagnosis of malaria in individuals with HIV/AIDS and receiving cotrimoxazole prophylaxis.

In conclusion, the sensitivity of mRDT in the diagnosis of asymptomatic malaria (and perhaps symptomatic malaria) in this cohort is reduced but its diagnostic value is retained in the specificity, PPV and NPV.

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Limitation of study

Cotrimoxazole naïve HIV infected children would have been more apt as controls for the study but ethical consideration would not permit the withholding of a drug of proven benefit to deserving patients.

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