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COMPARATIVE STUDY OF MODIFIED CENTRIFUGED BLOOD SMEAR, PERIPHERAL BLOOD SMEAR AND RAPID DIAGNOSTIC TEST METHODS IN THE DIAGNOSIS OF MALARIA INFECTION

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ABSTRACT

The gold standard for malaria diagnosis is microscopy with peripheral blood smear (PBS). Rapid diagnostic test (RDT) is a substitute especially where trained personnels are not available. Adoption of methods which improve the accuracy of malaria diagnosis will reduce inappropriate use of new expensive antimalarial treatments. The aim of this study was to compare the modified centrifuged blood smear (MCBS) as an alternative and more sensitive technique in malaria diagnosis. Venous blood was collected from 140 symptomatic subjects and subjected to three techniques for the diagnosis of malaria; PBS examination, MCBS examination, and RDT (CareStart). The MCBS method detected infection in 40% of the subjects examined, followed by PBS (36%) and RDT (20%). MCBS had a sensitivity of 92% and specificity of 90%, while PBS recorded a sensitivity of 71.4%, specificity of 80%, PPV of 80% and NPV of 82%. RDT (CareStart) had a sensitivity of 43%, specificity of 95%, PPV of 85.7% and NPV of 91.4%. Addition of the centrifugation step in MCBS increased the sensitivity of the peripheral blood smear method from 71.2% to 92%, and also its specificity from 80% to 90%. This study has confirmed that MCBS is a more sensitive, easy to perform and affordable technique in the routine diagnosis of malaria.

KEYWORDS: Malaria, Modified centrifuged blood smear, Peripheral blood smear, Rapid antigen detection.

BACKGROUND
Malaria debilitates the active population and the resources of the affected countries limiting their ability to contribute to socioeconomic development. In Africa, about US $12 billion is expended each year on increased healthcare cost, loss of manpower and negative effect on tourism (Worrall *et al.,* 2005).

Effective management of malaria requires prompt and accurate diagnosis. Light microscopic examination of peripheral blood smears has been very useful because it is less expensive, allows species identification and quantification (Moody *et al.*, 2012). However, the adoption of light microscopy as a gold standard method has been queried due to false negative result at low parasitaemia levels (Coleman *et al.*, 2006). Malaria can also be diagnosed with antigen-based diagnostic test kits. This study was to compare the efficacy of modified centrifuged blood smear (MCBS) to peripheral blood smear and rapid antigen diagnostic test in the diagnosis of malaria.

MATERIALS AND METHODS
Study Area
This study took place at St. Joseph's Hospital, Ikot Ene, Akpabuyo L.G.A, Cross River State, Nigeria. Ikot Ene is a rural community in Akpabuyo L.G.A which lies between latitude $4^o 5'$ and $5^o 40'$ and longitude $8^o 25'$ and $8^o 32'$ East. It lies within the rain forest belt of Southern Nigeria and shares the Atlantic coastline with Bakassi to the East and Rebulic of Cameroon to the West. Akpabuyo has a population of 2713,395 at the 2006 census. The people of Akpabuyo are predominantly fishermen and farmers.

Subjects, study design and duration
This was a cross sectional study of patients of all ages and sexes attending the outpatient departments of the hospital. The study was carried out from July to October, 2016.

Inclusion Criteria
Patients of all age groups and sexes with symptoms of malaria and attending various outpatient departments or on admission in the hospital were enrolled.

Exclusion Criteria
Patients who were taking antimalarial drugs or who had treated malaria within two weeks prior to the study were excluded.

Ethical Clearance
Ethical clearance was sought and obtained from the research ethical committee of the hospital. Informed consent was duly signed by all patients included in this study.

Sample Processing
Peripheral blood smear: Identification of malaria parasite for peripheral blood smear was done by Giemsa staining technique and calculation of parasite density by World Health Organization (WHO) criteria (WHO, 2010).

Rapid antigen diagnostic test procedure was done as per manufacturer's instructional manual. Carestart (RDT) was used because of its reported high sensitivity and specificity of 98% and 97.5% respectively.

Modified Centrifuged Blood Smear (MCBS): Briefly the blood was collected into heparinized capillary tubes, sealed with plasticine at one end, and was centrifuged for 5 minutes at 6000 rpm. The capillary tube was cut with a tube cutter above the buffy coat layer to retain a small plasma column. The buffy coat, small RBC column below the buffy coat and retained plasma column were placed on the slide, gently mixed and smear was made. The MCBS smears which had the same thickness as that of the PBS were then stained by Giemsa staining technique.

Data analysis
Data was analyzed using statistical package for social sciences (SPSS) version 20.0, IBM, Armonk, New York). Chi square test was used to determine the influence of age on infection. Results were expressed as mean ± standard deviation. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated. Results were statistically significant at probability value $p < 0.05$.

Table 1: Prevalence and intensity of malaria parasite infection with peripheral blood smear method according to age group of subjects examined.

Age group (yrs)	No. Examined	No.(%)with infection	MeanParasite Count. ±SD
1-10	10	10 (100)	7.6±4.0
11-20	16	8 (50)	5.0±4.5
21-30	86	28 (33)	5.0±2.8
31-40	12	0 (0)	4.0±2.8
41-50	8	2 (25)	5.0±2.0
51-60	8	2 (25)	6.0±5.0
Total	140	50 (36)	32.8±21.1

Table 2: Distribution of positive cases by each diagnostic method according to age groups of subjects examined.

Age Groups (yrs)	No. Examined	No.(%) positive with PBS	No.(%) positive with MCBS	No.(%) positive with RDT
1-10	10	10 (100)	8 (80)	10 (100)
11-20	16	8 (50)	8 (50)	2 (12.5)
21-30	86	28 (33)	28 (33)	14 (16)
31-40	12	0 (0)	2 (17)	0 (0)
41-50	8	2 (25)	4 (50)	0 (0)
51-60	8	2 (25)	6 (75)	2 (25)
Total	140	50 (36)	56 (40)	28 (20)

Table 3: Performance characteristics of MCBS, PBS and RDT.

Diagnostic methods	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
MCBS	92	90	82	96
PBS	71.4	80	80	82
RDT	43	95	86	91

RESULTS
Table 1 shows the prevalence and intensity of malaria parasite infection with peripheral blood smear method according to age group of subjects examined. Subjects within the age group of 1-10 years had the highest prevalence (100%) while subjects 31-40 years had the lowest prevalence rate of infection (16.7%). The difference was statistically significant (X^2=15.54, df=5, p=0.0006). Also, subjects 1-10 years had the highest mean parasite count of 7.6±4.0 while subjects 31-40 years had the lowest mean parasite count of 4.0±2.8. The distribution of positive cases by each diagnostic method according to age groups of subjects examined is shown in Table 2. The highest rate of infection was detected with modified centrifuged blood smear (40%), peripheral blood smear (36%) while rapid antigen detection kit was 20%. Their differences were not statistically significant (F=14.128, df=17, p=0.7349).

Table 3 shows the performance characteristics of MCBS, RDT and PBS. The sensitivity and specificity of PBS was 71.4% and 80% when compared to MCBS. The positive and negative predictive values were 80% and 82% respectively. RDT when compared with MCBS was 43% sensitive, 95% specific, with a positive and negative predictive values of 85.7%, and 91.4% respectively. The sensitivity of MCBS when compared to PBS was 92% and specificity was 90%. The positive and negative predictive values were 82% and 96% respectively.

DISCUSSION
The accuracy of peripheral blood smear examination (PBS), modified centrifuged blood smear (MCBS) examination and rapid diagnostic test (RDT) were compared among the subjects using PBS as the gold standard method.

Peripheral blood smear (PBS) recorded a sensitivity of 71.4% specific of 80%, PPV of 80% and NPV of 82%. These were almost similar to that reported elsewhere by Shambhavi Singh *et al.*, 2016 and Bhandari *et al.*, 2008. The low sensitivity of PBS in this study reveals the limitation of PBS examination as gold standard method in research and in routine clinical practice. Indeed, these findings justifies why clinicians do not take negative microscopy results as truly negative (Zurovac*et al.*, 2006; and Reyburn *et al.*, 2007).

Rapid diagnostic test kit (RDT) had a sensitivity of (43%), specificity of 95%, PPV of 85.7% and NPV of 91.40%. The sensitivity of RDT was unacceptably low as compared with results reported elsewhere (Mouatcho and Dean Goldring, 2013). The factors which may be responsible for the low sensitivity of RDT include inappropriate storage and transportation, low intensity of infection and inappropriate use of antimalarial. This sensitivity is below the sensitivity limit of >90% necessary for malaria diagnosis in routine practice (WHO, 2003).

As shown in Table 3, it was observed that centrifugation increased the sensitivity of PBS from 71% to 92%, specificity from 95% to 98%. These were similar to previous works reported by Shambhavi Singh *et al.*, 2016 and Bhandari *et al.*, 2008.

This report shows that 29% and 47% of subjects who were negative for both PBS and RDT were diagnosed of malaria by MCBS microscopy. The results were slightly higher than that reported in another study (Bhandari *et al.*, 2008). This showed that 13.21% of patients who reported as negative by PBS were actually negative by MCBS microscopy.

The influence of age on the methods used was statistically significant (p=0.001). Although PBS microscopy has limitations (Payne 1988), including the low sensitivity reported in this study, it is still useful in estimating the parasite density and for detecting other human plasmodium species (Abeku *et al.*, 2008).

In malaria diagnosis, Modified Centrifuged Blood Smear microscopy has demonstrated a better sensitivity when compared with PBS and RDT (CareStart). Based on this finding, MCBS may be suitable for screening malaria in routine practice provided there are well trained personnel, adequately equipped malaria diagnostic laboratory and both internal and external quality control system.

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