Evaluation of Malaria Prevalence Using Microscopic Examination and Histidine Rich Protein-2 Rapid Diagnostic Test in Anambra State, Nigeria

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Citation  

Abstract  
Malaria is a disease of severe public health importance and Nigeria suffers the world’s greatest malaria burden. This hospital-based study was designed to comparatively evaluate malaria prevalence using microscopy and Rapid Diagnostic Test (RDT) in Anambra, Nigeria. Four hospitals in Awka, Anambra state were chosen for the study. Blood samples of 322 patients who attended the hospitals were examined for malaria parasite infection using both Giemsa-stained thick film microscopy and Histidine-rich protein 2 Rapid Diagnostic Kit. Of the 322 patients enrolled, 194 (60.2%) tested positive by microscopy and 199 (61.8%) by RDT. Utilizing microscopy as the gold standard, the RDT showed a high sensitivity and specificity value of 100% and 96.1% respectively. The positive predictive value (PPV) of this diagnostic method was 97.5% while the negative predictive value (NPV) was 100%. There was an excellent agreement between the two methods as the Kappa value was 0.984. The difference in the two methods showed statistical significance. Based on our findings, the diagnostic performance of RDT was good. Hence, in the absence of good quality microscope and in emergency situation where quick diagnosis and treatment is required, RDT is an alternative and reliable method for diagnosis of malaria in Awka.

1. Introduction  
Malaria is a major cause of morbidity and mortality in Nigeria especially among children and pregnant women. It is a debilitating disease that affects the physical and economic well-being of people living in endemic areas of sub-Saharan Africa [1]. It still
remains the most important parasitic disease in the world. Apart from children and pregnant women, it also poses a risk to travelers and immigrants with imported cases increasing in non-endemic area [2]. According to the World Health Organization, 40% of the total global population is at risk of malaria infection. An estimated 3.4 billion people is at risk of malaria, of which 1.2 billion is at high risk. Ninety percent (90%) of all malaria deaths occur in sub-Saharan Africa. Majority of these deaths do occur among children living in Africa, where a child dies every minute from malaria [3].

Effective control and subsequent elimination of Malaria will in no doubt depend on efficient and accurate diagnosis. Prompt and accurate diagnosis is an essential component of Malaria control strategies and enables the effective management of febrile patient [4]. The global burden of malaria has spurred interests in developing diagnostic strategies that will be effective not only in resource limited areas where malaria has substantial burden on society but also in developed countries where expertise in malaria diagnosis is often lacking [5]. For several years, it has been common practice to base diagnosis of malaria mainly on clinical signs and symptoms in health facilities across Africa due to the scarce availability of laboratory facilities and the high mortality of malaria in young children [6]. The current WHO recommendation has switched to the systematic testing of all fever cases [7]. Presently, microscopy and Rapid Diagnostic Test (RDT) are the most widely used confirmatory test for malaria diagnosis in endemic areas. The principle of RDT method stems from detection of specific antigens (histidine, aldolase, and parasite lactate dehydrogenase) of malaria parasites in human blood. Microscopy provides the gold standard/reference for malaria diagnosis. However, it is time-consuming and requires trained personnel to perform it. On the other hand, RDT enables rapid diagnosis and treatment and requires little or no training to perform. It has become increasingly necessary to keep investigating the accuracy of these diagnostic methods by comparative evaluation especially as the world targets malaria elimination. Therefore, the aim of this study is to comparatively evaluate malaria prevalence using microscopic method and Rapid Diagnostic Test (RDT) in Anambra, Nigeria. Our findings will validate the usefulness of both diagnostic methods. Moreover, it will have implications in informing physician and patient choices in malaria diagnosis.

2. Materials and Methods

2.1. Study Area

The study was carried out between May to July 2015 in Awka, Anambra State, Southeast Nigeria. The geographical coordinates of Awka are Latitude 6°12’25”N and Longitude 7°04’04”E. The town is sited in a fertile tropical valley but most of the original rain forest has been lost to urbanization. The climatic condition of the town is characterized by two distinct seasons, wet season (from April to October) and dry season (from November to March). Harmattan winds blow for about four to six weeks between December and January. The temperature in Awka is generally 27-30°C between June and December but rises to 32-34°C between January and April, with the last few months of the dry season marked by intense heat.

According to the last census conducted in Nigeria in 2006, the estimated population of Awka stood at 301,657. Majority of the inhabitants are civil servants, business men and women and students because of presence of a public university in the city. There are many health facilities including clinics, hospitals, maternity homes, laboratories and patent medicine vendors (PMV) scattered across the city.

Figure 1. Map of Awka town, Anambra state, Nigeria showing location of study sites.

2.2. Study Design

The study population consists of patients who attended the selected private and public health facilities during the study period. The study population was made up of patients that attended private and public hospitals in Awka during the study period and who are residents in Awka. These included pregnant women, children, students etceteras. A two-stage cluster sampling technique was employed to select the sample size. First, four out of the twenty-six registered hospitals in Awka were chosen systematically. Second, eighty-one participants were selected randomly from each of
the chosen hospital. The sample size was determined using the Cochran formula \[n_0 = (t^2 \times p \times q)/d^2\]. Where \(n_0\) = required return sample, \(t\) = value for selected alpha level of 0.05, \(p = \) estimated prevalence, \(q = 1-p\) and \(d = \) acceptable margin of error for proportion being estimated, the minimum being 0.05. A sample size of 322 was determined, and 322 blood samples were taken.

### 2.3. Sample Collection

The inclusion criteria adopted was those who have not yet taken any antimalarial two weeks prior to blood collection, while those who have taken within two weeks before sample collection where excluded. This was done to rule out any effect of the drug on the malaria diagnostic outcome. Two milliliters (2mls) of blood was collected from each patient under aseptic conditions. This was immediately transferred into sample bottles containing anticoagulant (EDTA) and mixed thoroughly to avoid clotting. The samples were used to carry out both microscopy and RDT in parallel. The brand name of the RDT kit used was Carestart™ malaria antigen test kit with CAT number G0141, lot number MO2H5 and 25 test strips in the cassette form. This was made in USA by Access Bio company and is specific for detection of histidine rich protein 2 (HRP2) antigens of Plasmodium falciparum only. The test was carried out using the guidelines from Nigeria Federal Ministry of Health [9]. Thick blood film preparation as described by Cheesebrough [10] was used for microscopic examination.

### 2.4. Ethical Consideration

The study was conducted in accordance with the Declaration of Helsinki and received approval from the Ethics Committee (CNER) of Chukwuemeka Odimegwu Ojukwu University Teaching Hospital, Amaku, Awka, Anambra state (No. VO1.1020/AA/COOUTH). The study was explained to participants, and informed written consent for each patient, or parent in the case of children, was obtained before enrolment into the study.

### 2.5. Statistical Analysis

Data analysis was done using statistical package for social science (SPSS) version 21. A \(P\)-value of <0.05 was considered statistically significant.

### 3. Results

Of the 322 subjects sampled in the four hospitals, 91 (28.2%) were pregnant women, 72 (22.4%) were children while 159 (49.4%) were other population which included students, older men and women (Table 1). Divine hospital recorded the highest number of subjects which was 135 (41.9%) while Ikechukwu maternity recorded the least number which was 35 (10.9%).

<table>
<thead>
<tr>
<th>Sample Population Structure</th>
<th>Divine No. (%)</th>
<th>Trinity No. (%)</th>
<th>Regina Caeli No. (%)</th>
<th>Ikechukwu Maternity No. (%)</th>
<th>Total No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant</td>
<td>43(47.2)</td>
<td>7(7.7)</td>
<td>6(6.6)</td>
<td>35(38.5)</td>
<td>91(28.3)</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children</td>
<td>33(45.8)</td>
<td>21(29.2)</td>
<td>18(25.0)</td>
<td>0(0)</td>
<td>72(22.4)</td>
</tr>
<tr>
<td>Others</td>
<td>59(37.1)</td>
<td>33(20.8)</td>
<td>67(42.1)</td>
<td>0(0)</td>
<td>159(49.4)</td>
</tr>
<tr>
<td>Total</td>
<td>135(41.9)</td>
<td>61(18.9)</td>
<td>91(28.3)</td>
<td>35(10.9)</td>
<td>322(100)</td>
</tr>
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</table>

The Figure 2 highlights the comparison of malaria prevalence using microscopy and RDT. Of the 322 subjects sampled, 194 and 199 were positive for microscopy and RDT respectively. These gave a prevalence rate of 60.2% and 61.8% for microscopy and RDT respectively. The difference in the two methods was statistically significant (\(p=0.025\)).

![Figure 2. Comparison of Malaria Prevalence in Sample size Using Microscopy and RDT.](image)

Table 2 summarizes the performance effectiveness of microscopy and RDT in malaria diagnosis. The RDT showed 194 true positive results, which corresponded with the sensitivity value of 100% when compared with microscopy.
result. The specificity of the RDT kit was 96.1% corresponding with true negative result of 123. The RDT showed 5 additional positive results which were negative in microscopy thus making them false positive. True positive (TP) =194, True Negative (TN) = 123, False positive (FP=5) False Negative (FN)= 0, sensitivity=100%, specificity= 96.1%, positive predictive value (PPV) = 97.5% Negative predictive value (NPV) = 100% Kappa value= 0.984.

<table>
<thead>
<tr>
<th>Table 2. Performance Effectiveness of Microscopy and RDT in Malaria Diagnosis.</th>
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<tr>
<td><strong>Microscopy</strong></td>
</tr>
<tr>
<td>+ve</td>
</tr>
<tr>
<td>RDT</td>
</tr>
<tr>
<td>-ve</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

4. Discussion

The result showed significant difference in the prevalence of malaria between the two methods; microscopy and RDT. The slightly higher percentage of malaria prevalence with RDT may be attributed to antigenic persistence. It is possible that some of the subjects might have treated themselves for malaria before the test. Such persons may have residual malaria antigens, which can test positive with rapid test kit. According to Murray et al. [11] the HRP-2 antigen is known to possibly persist at detectable levels for more than 30 days, after the symptoms have disappeared, and the parasite forms that cause the disease have been cleared from the patient blood. This observation is in line with previous comparative studies between RDT and microscopic malaria diagnostic test which shows that RDT usually reveals higher prevalence compared to microscopy. In 2010, Fancony et al., [12] reported a prevalence rate of 60% for microscopy and 72.8% for the RDT used. Batwala et al. [13] also reported a prevalence rate of 18.7% for health centre microscopy, 15.7% prevalence for expert microscopy and prevalence rate of 36.7% with RDT in their study. Amadi et al., [14, 15] reported prevalence rate of 14.7% for microscopy while the RDT showed prevalence rate of 19.5%.

On the hand, our findings were in disagreement with the findings of Sheyin and Bigwan [16] who showed a positive rate of 52.9% for microscopy and 42.6% for RDT. Vanderjagt et al. [17] also reported a positive rate of 20% for microscopy and 4% for RDT. Our findings also differed with reports from Xiaodong et al. [18], Beyene et al. [19], Brown and Azike [20] and Adesami et al. [21]. These authors recorded high percentage of positive rates for microscopy when compared with RDT in their studies. These may be attributed to low parasitemia (low parasite density), deterioration of the kit due to storage condition.

Although microscopy is considered a gold standard, the present study shows that the RDT had an overall sensitivity of 100% and specificity of 96.1% among the study population. This sensitivity was in line with the WHO [4] prescribed sensitivity of over 90% for an acceptable RDT for malaria. The result collaborates with that of Samane et al. [22] that reported 98.5% sensitivity and 100% specificity. Our results though fairly higher compares favourably with that of the other studies like Singh, Bechem et al. [23], Oguonu, and Okafor [24], and Ansah, et al. [25] and Ben-Edet et al. [26]. Variations in test sensitivity between these studies maybe due to observer variations, differences in the types of RDT kits used, variations in epidemiologic characteristics of the study population, level of parasitemia, test methodology and skill of technician. Nevertheless, the present study like earlier studies in Nigeria [24, 27] further confirms the efficiency of the RDT as alternative method for rapid diagnosis of malaria in an endemic region. The RDT had high positive predictive value (97.5%) meaning that almost all the patients will be correctly diagnosed as positive for malaria and avoids unnecessary treatment that could foster drug resistance. The 100% negative predictive value means that it was reliable in ruling out malaria among positive cases.

5. Conclusion

The RDT kit used (Carestart™) has shown good sensitivity and specificity when compared with microscopy. This underpins RDT as a veritable tool for diagnosis of malaria in the study area, can serve as an acceptable alternative to routine microscopy for diagnosing malaria cases. In view of the performance of the RDT in malaria diagnosis, it is vital that all health facilities have supplies of this RDT kit for emergency cases that require rapid testing and short turnaround time.

Acknowledgments

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Conflicts of Interest

The authors declare no conflict of interest.

References


