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Diagnostic Accuracy of Malaria Microscopy in the Highlands of Central Kenya: Implications for Proper Treatment

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Abstract

Over-diagnosis of malaria is a problem in many African countries and appears to be occurring in the highlands of central Kenya. Blood-smear microscopy, the standard technique for malaria diagnosis in this area, relies heavily on technician skill. Hence, Rapid Diagnostic Tests (RDTs) are increasingly considered the gold standard for diagnosis, but are often not available in resource-constrained settings. We compared the results of microscopy and RDTs performed on finger-prick blood samples from 250 patients referred to laboratory malaria testing at one private and two government health facilities in/near Meru, Kenya. Across the three sites, 97.1%-100% of microscopy-diagnosed Plasmodium-positive samples were found negative by RDT. Of the three study sites, the government district hospital had the highest microscopy-based positive rate (27.3% as compared to 5.9% and 9.3% in the other two settings). These results indicate alarming levels of inaccurate malaria diagnosis in the Meru region. Many factors may play a role in this phenomenon, and it is likely that a “systems level” approach is necessary to remedy this problem.

Key words: Malaria, Diagnosis Accuracy, Kenya, RDT, Microscopy, plasmodium

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Introduction/Background

Malaria is one of the most prevalent infectious parasitic diseases in the world. In 2010, the World Health Organization reported 216 million cases of malaria and over 655,000 deaths. Though approximately half the world population is at risk, over 90% of cases occur in sub-Saharan Africa (World Health Organization World Malaria Report, 2009; Murray et al., 2012). That region experienced a 30% decline in deaths from 2004 to 2010, but remains the global epicenter of the disease (Murray et al., 2012). In 2009, 67% of the Kenyan population of 38.6 million was at risk for malaria, with over 8 million cases of malaria being reported annually (WHO World Malaria Report, 2009).

While it is undeniable that malaria infection is a serious problem in sub-Saharan Africa, recent studies suggest that the actual number of malaria cases may be lower in some locations than the reported incidence suggests. A prospective study in the Sudan showed a rate of false-positive malaria diagnoses of 75.6% (Salwa et al., 2009). A similar study in Tanzania reported a false-positive rate of 75.1% and linked misdiagnosis to increased patient mortality due to failure to treat alternative causes of severe infection (Reyburn et al., 2004). Furthermore, a study of seventeen hospitals across Kenya showed that revised clinical practice coupled with improved laboratory diagnostic techniques can save health systems large sums of money (Zurovac et al., 2006). Thus, while malaria continues to be a major health concern in many parts of Africa, unnecessary anti-malarial treatment and lack of treatment of other, overlooked infections may be wasting valuable healthcare resources, increase the development of resistant malaria infections, and lead to excess patient mortality from other febrile illnesses.

Over-diagnosis of malaria that has been noted in other areas of the world may also be occurring in the Meru region of central Kenya. Malaria in Kenya is typically restricted to below 1600m, though epidemics have been known to reach higher elevations (Arness et al., 2003). The elevation of the Meru region varies from ~1000-2000m, suggesting that *Plasmodium* transmission might not be widespread because of limited distribution or survival of *Anopheles* mosquito vectors. Local clinics and hospitals, however, report a high number of malaria cases as well as treatment of individuals year-round. Maps published by the Kenyan Ministry of Health and MARA (Mapping Malaria in Africa, Figure 1) show the presence of seasonal malaria in Meru. This discrepancy between the predicted incidence and seasonality and the reports of local practitioners warrants a detailed surveillance-based investigation.

In the highlands of central Kenya the common malaria *Plasmodium* parasite species are *P. falciparum* and *P. vivax*, both transmitted by *Anopheles* species mosquitoes (Arnss et al., 2003; Baliraine et al., 2009). Parasite transmission and the geographic range of malaria is affected by ecological factors such as humidity, altitude (a proxy for ambient temperature), density and movement of susceptible humans, and availability of suitable mosquito breeding sites (Malakooti et al., 2011). Like much of Kenya, the Meru region experiences two rainy seasons, a short one in November and an extended one lasting from late March through May. In areas like Meru where environmental indicators suggest lower-level risk and epidemic or seasonal disease patterns, accurate testing of suspected malaria is especially important, because presumptive diagnoses (either positive or negative) are less likely to be accurate. Such testing could reduce unnecessary treat-
ment for malaria, thereby reducing costs and improving accuracy of diagnosis. Accurate testing would allow for recognition and proper treatment of non-Plasmodium infections that produce febrile symptoms.

In addition, anti-Plasmodium drugs can be toxic. Several anti-malarials are known to cause adverse reactions in patients, though the cost-benefit equation differs when they are used for treatment versus prophylaxis (ALKadri, 2007). Each medication has contra-indications and cumulative toxicity can result in serious complications. Though no formal studies to measure adverse reactions to anti-malarials have been conducted in Merugion, local physicians have anecdotally reported cases of severe liver failure and other conditions they attribute to cumulative toxicity, perhaps from over-diagnosis of malaria.

There are several methods currently being used in Kenya to diagnose malaria. Presumptive treatment (PT) is used in some high-transmission areas where anti-malarial treatment is provided to any patient presenting with a fever, without a laboratory confirmation. Many areas employ more stringent clinical symptom-based diagnoses. Alongside clinical diagnoses many health facilities also employ basic laboratory capacity. The least-expensive and most common laboratory diagnostic tool involves microscopic examination of blood on slides to detect, count and identify Plasmodium species. Results typically are available within an hour depending on the daily workload. When multiple technicians with ample time undertake microscopic diagnoses, this can be the gold standard of malaria diagnosis. In many instances, however, equipment is limited or of low quality, confirmatory test-

ing absent and examination time is constrained by high patient volumes. Furthermore, microscopy technicians who believe that patients are being treated presumptively for malaria regardless of blood film results may be less motivated to properly examine the slide.

Rapid Diagnostic Tests (RDTs) are increasingly being used to diagnose malaria, especially in low-resource settings (Kamau, 2007). RDTs typically consist of a small plastic cassette with an attached membrane test strip. The test strip is impregnated with monoclonal antibodies for the target Plasmodium antigen, and immunochromatographic assay reveals coloured test lines, indicating infection or non-infection. These results are typically available in 5-20 minutes with minimal human interpretation or training needed. There are now over twenty different manufacturers of malaria RDTs and over sixty different products available (Kamau, 2007) with sensitivity and accuracy comparable to microscopy. RDTs are currently not widely used in Kenya (WHO World Malaria Report, 2011), but a plan for nationwide distribution was announced in November 2012 by the Ministry of Public Health and Hygiene (Musliime, 2012).

Accordingly, we undertook a study to evaluate the accuracy of malaria diagnosis in and around Meru, a region that experiences seasonal and spatially variable risk. The objective was to evaluate the accuracy of diagnosis of malaria by comparing the number of malaria cases diagnosed by microscopy with those determined by RDT. Secondly, we analyzed patient demographic data for patterns in age, gender, clinical symptoms, and elevation of patient residence.
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Materials and Methods

During one week in mid-June 2011, RDT and microscopy results were collected from patients at three health facilities in and around Meru. A private hospital, a large government hospital and a small government dispensary were chosen for the study. Meru District Hospital is a large government facility with 306 beds and a diagnostic laboratory with 6-10 laboratory staff present at a given time. The Kinoru dispensary, a small, government clinic located just off the main Meru intersection, has a simple diagnostic laboratory with one or two technicians who oversee all testing. St. Theresea’s Kiirua Mission Hospital is a private health facility located ∼30 km into the hills north of Meru, with two laboratory technicians present at most times.

This study was undertaken as part of quality control/quality assurance comparing the use of RDTs and slide microscopy under the mandate of the Kenya Methodist University to provide community health surveillance in the Meru region. Although human subjects were not directly involved in this laboratory testing quality control project, approval for the study by the Kenya Methodist University Institutional Review Board was obtained. No data allowing for individual identification of participants was collected.

Patients included in the study had visited one of the three participating health facilities, had been seen by the attending physician, and referred to the microscopy laboratory for malaria tests as part of normal hospital practice. A total of 259 patients were consented to participate in the study, and the analysis included 128 at Meru District Hospital, 68 at the Kinorudispensary and 54 from the St. Theresea’s Mission Hospital. Patient age, gender, and region of residence were recorded. There were no asymptomatic controls compared for this study.

Those patients for whom a malaria slide test was recommended proceeded to the laboratory as directed by the attending physician. Standard finger-prick blood samples were obtained by a hospital technician for microscopic evaluation. An additional drop of blood from that finger-prick was applied to an RDT kit. Our study used SD Bioline antigen RDTs that differentiate between P. falciparum (“Pf.”, targeted by HRP-2 antigen) and the other Plasmodium species (P. vivax, P. malariae, and P. ovale, collectively labeled “Pan”). Slides were evaluated by the laboratory technicians of that hospital using their normal procedures, while RDTs were evaluated by a member of the study team. The readers of each method were effectively “blinded” to the results of the other test. The samples were taken from 9am to 5pm over the course of 2-3 consecutive days at each site.

In addition to the blood sample, personal information was recorded for eventual association with laboratory tests including age, gender, village of residence, and chief complaints that brought the patient to the hospital. No data to identify individual patients were recorded.

Approximate elevations of the residences were acquired by Google Earth. About 90% of the villages of residence were identified and assigned an approximate elevation. When a village was located, the elevation of the primary school was used as a default value for all residents of that village.
Results

A total of 259 blood samples were tested by using both methods, but 9 were discarded because of unclear RDT readings. Of 250 valid comparisons, only one was RDT-positive for *Plasmodium* infection (Table 1). That one sample was also microscopy-positive. There were no samples that were microscopy-negative and RDT-positive. However, 44 samples were microscopy-positive but RDT-negative. An additional 205 samples were both RDT-negative and microscopy-negative.

The percent of cases determined to be positive by microscopy varied by facility (Table 2). The Kinoru dispensary and Kiirua Mission Hospital exhibited 5.9% and 9.3% microscopy-positive prevalence, respectively, while at Meru District Hospital the proportion was 27.3%. At both the Kinorudispensary and Kiirua Mission Hospital all of the microscopy-positivesamples were found to be negative by RDT. Meru District Hospital had the one RDT-positive sample. Thus, 97.1% of its microscopy-positives were unconfirmed by RDT.

Statistical analysis revealed a significant difference between the elevation of patient residences by study site (Table 3). The samples from Kiirua Mission Hospital and Kinoru Dispensary were not significantly different from each other, but the elevations associated with the Meru District Hospital samples were significantly lower than those of the other two facilities (p-values of <0.001 and 0.009 as compared against Kinoru dispensary and Kiirua Mission Hospital, respectively).

None of the self-reported clinical symptoms were significantly correlated with microscopy results at the 0.05 significance level.

Discussion

This study reveals a striking discrepancy between RDT and microscopy results at three healthcare facilities in the immediate Meru region. Our study found a high proportion of false positive microscopy-based diagnoses at all three study sites. Furthermore, the proportion of positive microscopy-based diagnoses at Meru District Hospital was three times as high as at the other two study sites.

Many factors that impact laboratory diagnosis could explain the high levels of inaccuracy and the difference among sites. These facilities generally have few laboratory staff, heavyworkloads, and the possibility of low quality or contaminated laboratory equipment or reagents. Technician awareness of the treatment provided to patients regardless of parasite testing results may lead them to allocate their energy toward other tests, or to tasks that have a stronger impact on treatment. Furthermore, the presence of interns at Meru District Hospital may have impacted microscopy diagnosis. Any efforts to remedy this situation must be comprehensive and consider systemic challenges to accurate testing as well as technological upgrades and enhanced technician training.

Other studies have shown that true positive malaria cases are more likely at lower elevations. We observed that Meru District Hospital’s patients reside at lower elevations than the patients of the other two facilities (Table 3). Perhaps the tendency for Meru District Hospital microscopy technicians to determine higher rates of malaria infection is due to knowledge about their patient’s residence. Future research could examine whether diagnostic laboratories located at borderline altitudes, serving populations both at higher and lower risk for infection, are more susceptible to misdiagnosis.
Conclusion

Misdiagnosis was a prominent feature of malaria laboratory testing at the three facilities in our study, and likely throughout the Meru region. The level of inaccurate laboratory results is alarmingly high, and could be resulting in adverse health impacts for the general population. Further studies are necessary to map true malaria risk and complete a more thorough analysis of malaria diagnosis procedures.

Of the three study sites, Meru District Hospital had the highest proportion of microscopy-based positive malaria diagnoses. The causes of the diagnostic inaccuracy are likely complex and systemic in nature, and comprehensive solutions should address facility infrastructure and staffing, supplies, and consider implementing new diagnostic technologies.

Acknowledgements

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References


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Figure 1: Duration of the Malaria Transmission Season in Kenya, Mapping Malaria in Africa (MARA)

Kenya: Duration of the Malaria Transmission Season

This map is a product of the MARA/ARMA collaboration (http://www.mara.org.za). 7 months 2001, Medical Research Council, PO Box 17120, Congella, 4013, Durban, South Africa. CORE FUNDERS of MARA/ARMA: International Development Research Centre, Canada (IDRC); The Wellcome Trust UK; South African Medical Research Council (MRC); Swiss Tropical Institute, Multilateral Initiative on Malaria (MIM) / Special Programme for Research & Training in Tropical Diseases (TDR), Roll Back Malaria (RBM); Malaria seasonality model: Tanser, F et al. 2001. Paper in preparation. Topographical data: African Data Sampler, WRI, http://www.igc.org/wri/ads/maps/ads/ads_idx.htm.
Table 1: RDT and Microscopy Results

<table>
<thead>
<tr>
<th>Location</th>
<th>RDT Negative</th>
<th>RDT Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>St. Theresa’s KiiuruaMission Hospital (N=54)</td>
<td>49</td>
<td>0</td>
</tr>
<tr>
<td>Microscopy Neg</td>
<td>49</td>
<td>0</td>
</tr>
<tr>
<td>Microscopy Pos</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td><strong>Kinoru Dispensary (N=68)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microscopy Neg</td>
<td>64</td>
<td>0</td>
</tr>
<tr>
<td>Microscopy Pos</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td><strong>Meru District Hospital (N=128)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microscopy Neg</td>
<td>92</td>
<td>0</td>
</tr>
<tr>
<td>Microscopy Pos</td>
<td>35</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2: Summary Table of RDT-Microscopy Comparison

<table>
<thead>
<tr>
<th></th>
<th>Kinoru Dispensary</th>
<th>St. Theresa’s KiiuruaMission Hospital</th>
<th>Meru District Hospital</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Samples</td>
<td>68</td>
<td>54</td>
<td>128</td>
</tr>
<tr>
<td>% Microscopy-Positive</td>
<td>5.9%</td>
<td>9.3%</td>
<td>27.3%</td>
</tr>
<tr>
<td>% RDT Positive</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.8%</td>
</tr>
<tr>
<td>% Microscopy-Positive not confirmed by RDTs</td>
<td>100%</td>
<td>100%</td>
<td>97.1%</td>
</tr>
</tbody>
</table>
Table 3: Mean Elevation of Patient Residence by Study Site

<table>
<thead>
<tr>
<th>Study Site</th>
<th>N</th>
<th>Mean Elevation (Meters)</th>
</tr>
</thead>
<tbody>
<tr>
<td>St. Theresa's KiiruaMission Hospital</td>
<td>48</td>
<td>1693.3</td>
</tr>
<tr>
<td>Kinoru Dispensary</td>
<td>64</td>
<td>1685.7</td>
</tr>
<tr>
<td>Meru District Hospital</td>
<td>107</td>
<td>1551.5*</td>
</tr>
<tr>
<td>Total Sample</td>
<td>219</td>
<td>1621.8</td>
</tr>
</tbody>
</table>

* Statistically significant at the p = 0.05 level