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Efficiency of Histidine Rich Protein II-Based Rapid Diagnostic Tests for Monitoring Malaria Transmission Intensities in an Endemic Area

Dokunmu Titilope Modupe¹,a), Olasehinde Grace Iyabo²,b), Oladejo David Oladoke¹,c), Olanrewaju Oladeji¹,d), Akinbobola Abisola¹,e), Adjekukor Cynthia Ufuoma¹,f), Yakubu Omolara Faith¹,g) and Adebayo Abiodun Humphrey¹,h)

¹Department of Biochemistry, Covenant University, Ota, Ogun state, Nigeria
²Biological Sciences Department, Covenant University, Ota, Ogun state, Nigeria

a) titilope.dokunmu@covenantuniversity.edu.ng
b) Corresponding author: grace.olasehinde@covenantuniversity.edu.ng
c)oladejodavid502@yahoo.com
d)oladeji9533@yahoo.com
e)johnsonabisola@yahoo.com
f)cynthiaadjes@gmail.com
g)omolara.yakubu@covenantuniversity.edu.ng
h)abiodun.adebayo@covenantuniversity.edu.ng

Abstract. In recent years there has been a global decrease in the prevalence of malaria due to scaling up of control measures, hence global control efforts now target elimination and eradication of the disease. However, a major problem associated with elimination is asymptomatic reservoir of infection especially in endemic areas. This study aims to determine the efficiency of histidine rich protein II (HRP-2) based rapid diagnostic tests (RDT) for monitoring transmission intensities in an endemic community in Nigeria during the pre-elimination stage. Plasmodium falciparum asymptomatic malaria infection in healthy individuals and symptomatic cases were detected using HRP-2. RDT negative tests were re-checked by microscopy and by primer specific PCR amplification of merozoite surface protein 2 (msp-2) for asexual parasites and Pfssp2 gene for gametocytes in selected samples to detect low level parasitemia undetectable by microscopy. The mean age of the study population (n=280) was 6.12 years [95% CI 5.16 – 7.08, range 0.5 – 55], parasite prevalence was 44.6% and 36.3% by microscopy and RDT respectively (p =0.056). The parasite prevalence of 61.5% in children aged >2 – 10 years was significantly higher than 3.7% rate in adults >18years (p < 0.0001, χ² = 60.45). RDT detected additional 29.6% asymptomatic cases but a lower specificity of 68.8% in symptomatic carriers. In 15 selected RDT positive samples, only 6 were positive by PCR and no gametocyte was detected. The results indicate that HRP-2 RDTs are a vital tool for understanding transmission dynamics and detecting immune-suppressed, recent and asymptomatic infections, thus crucial to tackle low level transmission and eliminating malaria in endemic areas.

INTRODUCTION

For decades, Plasmodium falciparum malaria caused great public health burdens globally, defying control efforts but in recent years, there have been success in reducing transmission, morbidities and mortality in different regions [1,2]. Hence global efforts now target eliminating the infection in different regions with an ultimate aim of eradicating the disease [3]. Achieving this goal of successful elimination in endemic areas require reliable epidemiologic data and close monitoring of transmission. In Nigeria, an estimated 110 million malaria cases and over 300,000 deaths are recorded per year [4]. A pooled analysis of epidemiological studies in Nigeria was used to estimate the past and current state of malaria transmission in Nigeria, showing the importance of community and National surveys, indeed
transmission rates are declining in Nigeria [5]. Rapid diagnostic tests (RDTs) and light microscopy are both recommended for malaria diagnosis in areas and countries that are eliminating malaria [3]. RDTs are very useful for epidemiological studies in endemic areas because they are simple, easy to use for field studies, requiring no skills and having different antigenic targets [6-9]. However, there are challenges of variable specificity, sensitivity, false positives, false negatives, availability, and quality of RDTs outcomes hence requiring continued monitoring. Studies have reported variable HRP-2 RDT tests based on age and infection rates, post-treatment persistence of parasite antigens, level of immunity and in different endemic settings [10, 11]. RDTs generally diagnose *P. falciparum* malaria with a sensitivity of >90% [8, 12-16], an advantage in endemic areas will be detection of asymptomatic parasitemia. Hence, HRP-2 RDTs could serve as a tool for mapping intensities of transmission in untreated population, because HRP-2 protein can be detected both in asexual parasites and from young gametocytes of *P. falciparum*. In *P. falciparum* infected red blood cells, merozoite surface protein 2 (*msp 2*) is a highly expressed; a study has reported the diversity of *falciparum* infections in this area, thus can reliably be used to confirm *P. falciparum* infections [17]. RDTs can solve problems of misdiagnosis at low parasitemia, and missed detection by microscopy which can be detected through parasite antigens [7, 9, 18]. After several years of implementing different control measures, transmission intensities would have declined but the intensities of transmission continue to vary in different communities and over time in Nigeria [4]. It is thus imperative to utilize fast, economical, consistent and accurate combined techniques for studying transmission dynamics in this endemic area during the pre-elimination phase. This study aims to improve on RDT methods for malaria diagnosis by evaluating the current rate of transmission in an endemic community using microscopy, RDT and polymerase chain reaction (PCR) for assessing transmission intensities in Ota, southwest Nigeria.

**MATERIALS AND METHODS**

The study was carried out in Ota, a metropolitan city in Ogun state, southwest Nigeria where malaria transmission occurs throughout the year but highest during the rainy season (April to October) when average rainfall is about 107.3 mm. The annual temperature ranges from 22.8°C to 39°C. The cross-sectional study was carried out in individuals participating in voluntary community health survey in Ota during the dry season. The inclusion criteria include: individuals with no history of taking antimalarial treatment two weeks prior to sampling, and informed consent to participate in the study. Exclusion criterion was prior treatment for malaria within two weeks before the test. Ethical approval was sought from the local authority and the community leader to carry out the study.

Laboratory test: Presence of malaria parasite was detected by histidine rich protein II (HRP-2) rapid diagnostic test (RDT) kits following the manufacturer’s instruction (Standard Diagnostic Inc. Hagal-Dong, Korea). Briefly, blood sample (10ul) was placed in the blood well of the cassette and few drops of the flushing buffer was also placed into the buffer well, the test was read within 15 minutes according to the manufacturer’s guide. Two lines at the control and test regions respectively indicates a positive result while a single line at the control region indicates a negative result. If no line appeared in the control region, the test was read as invalid. Thick smears were prepared for microscopy, dried, stained with Giemsa, and viewed under the microscope at 100x magnification by a trained personnel to determine presence of parasites. Negative slides were re-examined by an independent microscopist. Polymerase chain reaction amplification of block 3 region of *msp*-2 followed DNA extraction using Aidlab® tissue extraction kit, and methods previously described [17, 19] was used at a final volume of 25μl. Briefly, RNA extraction from blood spotted on filter paper was done using Promega® kit in RDT false positive samples, cDNA was synthesized using TRUEscript H- RTase/RNase inhibitor and 2X reverse transcription mix, RNA was incubated at 25°C for 10minutes, 42°C for 50 minutes and reaction terminated at 65°C for 15 minutes. Amplification of *Pf625* gene was done according to the method of Mlambo and colleagues [20] in a final volume of 25μl on Thermal cycler (BIORAD 2000). The PCR amplicons were resolved on a 2% agarose gel and visualized under UV transillumination for gel documentation. Data was analysed using SPSS for continuous data, while qualitative data was analysed using Microsoft Excel.

**RESULTS**

Overall, 280 individuals (100 symptomatic and 180 healthy participants) with mean age, 6.12 years (95% CI 5.16 – 7.08, range 0.5 – 55) were evaluated for the presence of malaria parasites in the study area. Figure 1 shows the age distribution of parasite prevalence in the study group. 91.2% malaria cases were recorded in children aged < 10 years and prevalence was highest in children aged >2 – 10 years (p < 0.0001, $\chi^2 = 60.45$) while older children and adults
accounted for only <10% cases. Malaria parasite was demonstrable in 125 blood smears with a prevalence rate of 44.6%. This was significantly higher than 36.3% prevalence rate by RDT (p = 0.056). In the positive cases, 38% were asymptomatic infection and 26.7% of this was detectable by RDT. In the blood films taken from symptomatic individuals, RDT sensitivity was relatively low, detecting only 53 of 77 (68.8%) positive cases with 2 invalid results.

**FIGURE 1.** Age distribution and parasite positivity rate in the study population. Children aged >2 – 10 years had the highest population and parasite prevalence during the study.

Microscopy negative and RDT positive (false positive results) were tested for true positivity using PCR. Fifteen successfully amplified samples showed 6 samples to be positive, a positivity rate of 40% for msp2 gene in the asymptomatic group, but there were no detectable gametocytes by RT-PCR in these samples. Figure 2 shows the gel image of PCR amplification for msp-2, of the 6 samples positive for msp-2, 3 distinct bands are seen while others were faintly seen. In these RDT false positive samples, parasitemia was not detected by microscopy. The parasite prevalence by RDT was re-calculated as corrected RDT parasite prevalence rate of 38.4%, this rate was similar to microscopy prevalence rate (p = 0.165, $\chi^2 = 1.92$).

**FIGURE 2.** Gel image showing amplified msp2 gene (gene size 380bp) in some microscopy negative samples [L = 1kilobase DNA ladder, 1 – 15 are samples, Lanes 2, 6, 8, are distinctly positive samples while lanes 1, 3, 9 were slightly positive].

**DISCUSSION**

Malaria transmission in highly endemic areas occur all year round but in recent times, there is declining transmission and associated deaths globally and in many endemic countries, due to adoption of artemisinin based combination therapies, distribution and use of long lasting insecticide treated nets, vector control and scaling up of other control measures [1, 3]. This trend applies also to Nigeria where geospatial data indicates that transmission rates continue to decline over many years of implementing malaria control, highlighting the importance of strategic control of malaria. The reported epidemiological survey in different communities across the country provides such evidence [5]. In this era of declining transmission, the success of malaria elimination will depend largely on accurate diagnosis.
and epidemiological data which are both crucial for targeted interventions of disease control [3]. Challenges of correctly estimating transmission rates which mitigates the control strategies in endemic areas can be overcome. Microscopy remains the standard for malaria diagnosis which requires highly skilled personnel. It however can be subjective, laborious, and sometimes present with problems of identifying artifacts as parasites. When malaria expert is unavailable or for field studies, RDTs are recommended. Our study compared HRP-2 RDT efficiency, sensitivity and reliability with microscopy for determining the transmission intensity of malaria in a metropolitan community in southwest Nigeria, and the contribution of asymptomatic infection to the transmission intensity. The present study reports a transmission rate of about 40% in symptomatic and healthy individuals in which the latter contributes more than half (26%) of the total infection. Malaria prevalence in this study was significantly higher than rates reported in hospital population from some southwest region [14-15] but similar to rates reported in other southwest and Northern Nigeria [16, 21-23]. The reasons for this are not farfetched, our study evaluated both healthy and hospital population thus revealing the intensities of malaria transmission in a general population. It is obvious that transmission intensities are very dynamic, changing with factors modulating transmission; such as climate, vector efficiency and location due to intensified control efforts [24-25].

Global reports on the status of malaria eradication indicate about 7 of the 43 endemic African countries with more than 25% population still at risk of malaria. Our reported rate is higher than that reported in countries in the African region and other regions outside the region working towards malaria elimination with very low rates e.g. < 10% in Papau New Guinea, Haiti etc, [2, 3]. Although, declining rates are reported in Nigeria over several years [5], our findings which is limited to a community in a sub-region of Nigeria showed a high transmission rate especially asymptomatic infection. Success of malaria eradication in endemic areas will benefit from a systematic review of prevalence rates using uniform diagnosis criteria such as RDT and microscopy used in the present study. It is also crucial to assess the effectiveness of these tools periodically to plan the needed strategies for different stages of elimination. True positivity of RDT was tested using PCR for msp-2 which was detectable in 6 samples, the other PCR-negative results may be due to recent infection detectable by persistent parasite antigens or presence of young gametocytes [6, 7, 18], although mature gametocytes were not detected in this study. A recent study reported that in this region, falciparum infections are multiclonal with high diversities within the msp-1, msp-2 and glurp genes. Multiplicity of infections has been linked to transmission intensities and is thought to also have important implications for epidemiology of drug-resistant parasites [17]. Although family specific alleles were not determined, by implication, our findings may be suggestive that transmission and resistant isolates are high in this region. Development of resistance in P. falciparum cause significant public health impact including increasing morbidity, mortality and reversal of successes of malaria control efforts [1-3]. Msp-2 detection in 40% false positive results by PCR further validates and corrects RDT outcomes. Detection of parasite antigen after clearance of infection by immunity which increases with malaria exposure, prior antimalarial use may explain possible reasons for RDT false positivity in the remaining samples. This sensitivity of RDT was >95%, while PCR-corrected prevalence of 38.4% was similar to microscopy prevalence rate (44.6%), this supports reports of high sensitivity but variable specificity reported in other studies [11-12, 14-15, 26], this falls within recommended rates by the World Health Organization [13].

Low level circulating asymptomatic infections which contributes significantly to malaria transmission is common in endemic areas [27-33]. Studies reports up to 30–50% cases of missed infections diagnosed by RDT and microscopy are detectable by PCR [28-29]. Our study confirms this as a major drawback for estimated malaria intensities in endemic areas due to limited resources for carrying out PCR, thus a poor reflection of the true transmission rates. Malaria control through artemisinin based chemotherapy has proved very effective in this area [34-35]; however understanding malaria dynamics, monitoring transmission in the pre-elimination stage in an endemic area is essential and will require combined control tools. Some antimalarials have potentials to reduce transmission e.g. sulphadoxine-pyrimethamine, artemisinin derivatives and 8-aminoquinolines e.g. primaquine, by reducing gametocytes with significant declines in transmission [36-42], but are not recommended in this area, hence it continues to sustain parasite transmission in endemic areas as well as other low endemic regions [26, 27, 29-31, 43]. About 4% gametocyte carriage rates have been reported previously in Nigeria [34]; current gametocyte carriage rates in Nigeria in healthy individuals are unknown. There are limitations of the present study: the true negativity of microscopy results were not determined, further studies would be carried out to validate this and improve the monitoring of transmission intensities, ultimately to reduce asymptomatic parasitic infection. Further studies are required to determine gametocyte prevalence and molecular markers of antimalarial resistance in the study area. Effective diagnosis and drug treatment will impact greatly on the risk of infection in the future and malaria elimination [4, 44]. In conclusion, malaria HRP-2 rapid diagnostic test kits have demonstrated high efficiency and may find usefulness in endemic areas to monitor low level
asymptomatic infection and for monitoring transmission intensities during the elimination stage in an endemic area such as in the present study area.

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