

Performance Evaluation of Plasmodium falciparum Histidine-Rich Protein 2 Rapid Diagnostic Test, when Compared to Microscopy

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Abstract

Introduction: In malaria diagnosis, a highly sensitive and specific test will ensure appropriate administration of antimalarial treatment, hence promoting a parasite-based diagnosis as recommended by the World Health Organization (WHO). The Global Malaria Program recommends that suspected clinical malaria could be confirmed using the quality assured Rapid Diagnostic Test (RDT) and microscopy diagnostic tools. This study was designed to assess the performance of Plasmodium falciparum Histidine-Rich Protein 2 (PfHRP2-RDT), with respect to age and parasite density.

Methodology: This study was carried out in the Bamenda Regional Hospital Laboratory, with 381 patients enrolled into the study by convenient sampling technique. A simple questionnaire, microscopy and PfHRP2-RDT techniques were used to collect data on sex, age, and malaria status of the study participants. Both descriptive statistics and analysis of variance were used for data analysis.

Results: Results by microscopy show that up to 68.55% (109/159) of the males and 41.89% (93/222) of the females were infected. About 55.44% of those infected were younger children (≤ 5 yrs) and young adults (>18 yrs to ≤ 35 yrs), with up to 68.81% of the infections being mild parasitemia. Results by microscopy and PfHRP2-RDT were not the same, and the difference between the daily variation in test results was significant at $P=0.0012$. With microscopy as the standard, the sensitivity, specificity, positive predictive value and negative predictive value of PfHRP2-RDT were; 100%, 92.75%, 94.26% and 100% respectively.

Conclusion: The microscopy technique indicated low specificity and positive predictive values. Hence, in order to ensure an effective parasite-based malaria diagnosis, a microscopy confirmatory test is recommended for every PfHRP2-RDT positive result.

Keywords: Plasmodium falciparum; Microscopy; Histidine-rich protein; Rapid diagnostic test

Introduction The sensitivity of a test, which is its ability to accurately identify the presence of the infectious agent is as important as the specificity, which accurately identifies the

absence of the infectious agents. In malaria diagnosis, a highly sensitive and specific test will ensure appropriate administration of antimalarial treatment, hence promoting a parasite-based diagnosis as recommended by WHO [1]. In malaria endemic settings, the rapid diagnostic test (RDT) and microscopy are suitable diagnostic methods for routine malaria clinical cases, which covers most of the microscopy and RDTs done in the public health sectors [2]. In fact, the Global Malaria Program recommends that suspected clinical malaria can be confirmed using the quality assured RDT and microscopy diagnostic tools [2]. That explains why malaria diagnostics with the largest impact on malaria control has been microscopy and RDTs [3]. However, these diagnostic techniques may be inappropriately used, due to inadequate laboratory support in malaria endemic areas where therapeutic management of febrile patients is frequently based on inaccurate clinical diagnosis [3]. Nonetheless, with proper quality control and quality assurance system, the microscopy method can be accurately used in diagnosing malaria as the cause of febrile illness. However, marked inadequacy in the quality control system may, amid other factors contribute to the recurrent impaired malaria diagnosis by the microscopy method reported even in hospital-based laboratories [4,5]. Therefore, there is need for a more convenient and less complicated procedure in malaria diagnosis. The malaria RDT is the current alternative which fits that need. Although RDT sensitivity reduces with reduced level of malaria parasitaemia ($<500/\mu\text{L}$ for *P. falciparum*), according to WHO, it should reach at least 95% in order to be a helpful diagnostic tool [6]. In order to conveniently rely on RDT as a necessary substitute for the microscopy technique, this study was designed to evaluate the performance of PfHRP2-RDT, in the Bamenda Regional Hospital Laboratory within the periods of April to June 2018. Specifically, this study was designed to assess the performance of PfHRP2-RDT, using microscopy as the standard.

Background literature

Factors like poor techniques in slide preparation, heavy work load, poor condition of the microscope, poor quality of laboratory supplies and insufficiently handled skilled microscopy will cause poor malaria diagnosis [7]. But with proper quality control and assurance system in place, microscopy can be used to quantify and identify malaria parasite species. In fact, it was reported that asexual parasites

can be detected by a skilled handling of the microscope at a density of <10 parasites per μL of blood [8]. However, the sensitivity reduces to <100 parasites per μL in field conditions [8]. Alternatively, the RDT procedure is less complicated, with generally cost-beneficial kits requiring very little to be effectively run. Although a few factors such as environmental conditions in the manufacturing process, may affect RDT performance [9,10]. RDTs generally require little operator training. Nonetheless, malaria parasites cannot be quantified and parasite species identified with RDT, it however prevents missed diagnosis of malaria or febrile illnesses with different etiologies [7]. Studies which considered microscopy as the gold standard found that RDT exhibited low sensitivity and high specificity [11,12]. In a malaria endemic zone, when compared to film microscopy the sensitivity, specificity, PPV and NPV of RDT were 82.2%, 100.0%, 100.0% and 34.3%, respectively, with a significant difference between both test methods [13]. Meanwhile in a hypo endemic zone, the sensitivity, specificity, PPV and NPV of RDT were 90.0%, 99.9%, 90.0% and 99.9%, respectively [14]. And in a meso endemic zone, the sensitivity, specificity, PPV and NPV of RDT were 91.0%, 65.0%, 71.6% and 88.1% respectively [14]. Studies have even shown that false positive RDT results are associated to high rheumatoid factor levels, leishmaniasis, hepatitis C, Schistosomiasis, toxoplasmosis, human African trypanosomiasis, dengue and Chagas disease [15,16]. Individuals with history of malaria and children were also found to be associated with false positive RDT results [17]. Due to low-density infection, sensitivity and PPV were low, in Swaziland, a low-transmission area [18]. A statistically significant association was found between malaria positivity rate and male, children below five years of age and those with fever more than 24 hours before diagnosis [18]. Although the sensitivity and positive predictive values of RDT were low, higher values were reported in patients with fever, as compared to non-febrile patients [18]. Consequently, the specificity of RDT and even its cost-effectiveness can be affected not only by the presence of some infections, but also by age, malaria endemicity, season and the presence of fever [14]. Malaria antigen target RDTs are immunochromatographic assays which uses monoclonal antibodies on a test strip, to detect malaria antigen in a small amount of blood. Histidine-Rich Protein 2 (HRP-2) which is specific to *P. falciparum* is the most frequent malaria antigen target in RDTs. Although HRP-2, has been shown to remain in the blood of the patient for weeks even after successful treatment, *Plasmodium falciparum* HRP-2-RDT is still considered a good laboratory test for malaria detection at low-level, in chronic cases [19]. It has even been reported that the sensitivity of HRP-2 tests was frequently greater than 90% [8,20]. RDTs are generally considered an effective diagnostic tool of malaria, which are easy to perform [21]. The highly sensitive and stable RDTs that detect the histidine-rich protein 2 (HRP2) antigen is recommended in endemic areas where *P. falciparum* is dominant [22]. PfHRP2-

RDT is also recommended even with the availability of RDTs which detects the enzyme parasite lactate dehydrogenase (pLDH), produced by all four human *Plasmodium* species [22]. But due to the persistence of HRP2 for several weeks after treatment, HRP2-based tests have been reported to show high number of false positives, resulting in low specificity [23,24]. Furthermore, the HRP2 protein has been reported to show variation in its repeat section which may be the cause for extensive variation in the sensitivity of HRP2-based RDTs [25]. According to another findings, RDT will be a useful substitute where there is high parasite density [13,14]. In fact, the HRP2-based RDT was shown to have higher sensitivity, as compared to microscopy in malaria diagnosis [26].

Conclusion: Based on the microscopy technique, there was a high malaria prevalence rate of 53.02% (202/381) among the study population, with 68.81% (129/202) of the infected cases being mild parasitemia. The difference between the daily results by microscopy and PfHRP2- RDT was statistically significant at $P=0.0012$. In the absence of mixed infections, the PfHRP2-RDT method has shown good sensitivity (100%), but relatively poor specificity (92.75%) with microscopy as the standard. Considering the good sensitivity, PfHRP2-RDT appears to be a suitable substitute for microscopy. However, judging from the negative predictive value (100%) and the positive predictive value (94.26%), a negative result proved reliable, but not a positive one. Also because of the low specificity (92.75%), a microscopy confirmatory test is recommended for every PfHRP2-RDT positive result. This study was however limited in that, the presence of other infections which could have affected the sensitivity/specificity of the PfHRP2-RDT was not tested. Additionally, the history on malaria infections in the patients was absent. For further studies, study participants should be screened for all possible current infections which could hinder the PfHRP2-RDT test specificity.

most commonly detected bacteria followed by *Campylobacter*. *Shigella* was most commonly detected in the 0 to 1 age group, followed by the 2 to 4 age group. Only one dual bacterial infection, which was in an adolescent patient, was detected.