INTRODUCTION

Malaria is preventable and treatable mosquito-borne illness. According to the world malaria report 2013, an estimated 3.4 billion people are at risk of malaria, of whom, 1.2 billion are at high risk and in high-risk areas, >1 malaria case occurs per 1000 population. Approximately, 90% of all malaria deaths occur in sub-Saharan Africa and as per the report, malaria killed an estimated 482,000 children under five year of age in 2012, i.e. 1300 children every day or one child every minute1.

Malaria is one of the leading public health challenges in Ethiopia. About 52 million people (68% of the population) live in malaria risk areas of the country. Plasmodium falciparum and P. vivax are the predominant malaria parasites distributed in most parts of the country. Weekly malaria surveillance database of the Amhara regional health bureau showed that malaria accounted for 22% of outpatient visits, 24% of hospital admissions and 10% of health facility deaths in the region2.

An essential component of malaria control and elimination strategies is prompt, accurate diagnosis and treatment (within 24 h of onset of illness). The process of diagnosis is initiated by a suspicion of malaria on the basis of a defined set of clinical criteria, which may vary with the level of malaria endemicity and the types of non-malaria fevers in the area. Diagnosis of malaria is therefore, confirmed by laboratory tests, either by blood film microscopy or rapid diagnostic test3.

The two malaria diagnostics tests: Microscopy and rapid diagnostic tests (RDTs) have the largest impact on malaria control in malaria endemic regions of the globe4. The Federal Ministry of Ethiopia also launched laboratory diagnosis programme for promoting use of either RDT or Giemsa microscopy for detecting suspected malaria cases at all levels of health facility (by microscopy....

Performance evaluation of rapid diagnostic test for malaria in high malarious districts of Amhara region, Ethiopia

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ABSTRACT

Background & objectives: Malaria is one of the leading public health challenges in Ethiopia. To address this, the Federal Ministry of Ethiopia launched a laboratory diagnosis programme for promoting use of either rapid diagnostic tests (RDTs) or Giemsa microscopy to all suspected malaria cases. This study was conducted to assess the performance of RDT and influencing factors for Giemsa microscopic diagnosis in Amhara region.

Methods: A cross-sectional study was conducted in 10 high burden malaria districts of Amhara region from 15 May to 15 June 2014. Data were collected using structured questionnaire. Blood samples were collected from 1000 malaria suspected cases in 10 health centers. RDT (SD BIOLINE) and Giemsa microscopy were performed as per standard procedures. Kappa value, logistic regression and chi-square test were used for statistical analysis.

Results: The overall positivity rate (PR) of malaria parasites by RDT and Giemsa microscopy was 17.1 and 16.5% respectively. Compared to Giemsa microscopy as “gold standard”, RDT showed 83.9% sensitivity and 96% specificity. The level of agreement between first reader and second reader for blood film microscopy was moderate (Kappa value = 0.74). Logistic regression showed that male, under five year of age and having fever more than 24 h prior to malaria diagnosis had statistically significant association with malaria positivity rate for malaria parasites.

Interpretation & conclusion: The overall specificity and negative predictive values of RDT for malaria diagnosis were excellent. However, the sensitivity and positive predictive values of RDT were low. Therefore, in-service training, quality monitoring of RDTs, and adequate laboratory supplies for diagnostic services of malaria would be crucial for effective intervention measures.
Several factors in the manufacturing process and environmental conditions may affect RDT performance. Manufacturers usually recommend 4–30°C as the optimal temperature range for RDT. In practice, exposure of RDTs to >70% humidity and/or >30°C temperature frequently occurs in the tropics like Ethiopia. Thus, quality control measures are important to ensure that the purchased RDTs meet the expected performances. The RDT product quality needs to be maintained through the delivery process to the periphery of the healthcare system. However, to be a useful diagnostic, RDTs must achieve >95% sensitivity.

A study in Ethiopia indicated that ParaScreen RDT exhibited high specificity (98.5%) and moderate sensitivity (47.5%) with a positive predictive value of 56.8% and negative predictive value of 97.6%. The overall slide positivity rate was 4.1% while ParaScreen RDT had positivity of 3.3% of those tested.

The performance of three multi-species (PfHRP-II/pan-pLDH and PfHRP-II/aldolase) RDTs (CareStart®, ParaScreen® and ICT Combo®) with ‘gold standard’ microscopy has shown that all three RDTs were equally sensitive in detecting *P. falciparum* or mixed infection (85.6%). RDT specificity was similar for detection of *P. falciparum* or mixed infection at around 92%. For detecting *P. vivax* infection, all three RDTs had similar sensitivity in the range of 82.5 to 85%. CareStart® had higher specificity in detecting *P. vivax* (97.2%) than both ParaScreen and ICTCombo.

The quality of malaria diagnosis be it Giemsa microscopy or RDTs needs to be monitored for accurate diagnosis and treatment. A study conducted in Amhara region showed that 52.6% of malaria patients were satisfied with malaria diagnostic services. No data has been documented on laboratory supplies and infrastructure for malaria diagnosis, work experience in microscopy and the performance of RDT in the Amhara region. This study was, therefore, conducted to evaluate the performance of RDT (SD Bioline) in the country (available during the study period) and influencing factors for quality of malaria diagnosis. Furthermore, associated risk factors for malaria cases were also assessed.

**MATERIAL & METHODS**

**Study design and area**

A cross-sectional study was conducted in 10 high burden malaria districts (Jawi, Ankasha, Metema, South Achefer, Gondar Zuria, Libokemkem, Fogera, Finoteselam town, Burie town and Mecha) of Amhara region from 15 May–15 June 2014. All the 10 districts are situated in the western sub-region with a total population of 1,455,890. The study was conducted in 10 health centers with a relatively high malaria patient flow in the above mentioned districts.

**Study population**

Patients who visited the 10 health centers for medical attention were considered the source population. Patients suspected for malaria by a clinician were the study population.

**Exclusion criteria:** Patients with a history of anti-malaria medication within 15 days of initiation of study were excluded.

**Sample size**

Ten districts were selected based on the high prevalence of malaria including one health center with expected high case load from each woreda (district). The overall prevalence of malaria in 10 districts was 9% per year. Therefore, the minimum sample size using Buderer’s formula was calculated based on both anticipated sensitivity and specificity as follows:

\[ n = \frac{Z_{1-\alpha/2} \times S_N \times (1-S_N)}{L^2 \times \text{Prevalence}} \]

\[ n = \frac{Z_{1-\alpha/2} \times S_p \times (1-S_p)}{L^2 \times (1-\text{Prevalence})} \]

Where, \( n = \) Required sample size; \( S_N = \) Anticipated sensitivity; \( S_p = \) Anticipated specificity; \( \alpha = \) Size of the critical region (1- \( \alpha = \) confidence level); \( Z_{1-\alpha/2} = \) The 95% confidence interval of standard normal deviate; and \( L = \) The desired precision.

According to the above two formulas sample size for sensitivity and specificity became 811 and 270, respectively. Out of the two, 811 was taken as the minimum sample size because taking larger sample size doesn’t affect the test specificity. The non-response rate and slide breakage (10%) with any possible transportation in and outside the laboratory was considered together making it 892. Finally, considering breakage of slides during transportation, damages of RDTs at health centers and similar malaria diagnostic service of health centers, the sample size was decided to be 1000 and distributed equally into 10 health centers.

**Data collection**

**Training of data collectors:** Training was given to medical laboratory personnel working at health centers, at
hospital, regional laboratory and to supervisors on data collection procedures. A structured questionnaire was used to collect data on demography and pertinent variables. Medical laboratory technicians were also interviewed for their experience in malaria diagnosis and each laboratory was also assessed and observed for furniture’s, equipments and other logistics. During the study period, the preceding three months RDT and blood film Giemsa microscopy results were also collected from each health center.

Blood sample collection: About 5 µl of blood for RDT and thin and thick blood films was collected aseptically from capillary of finger prick of each febrile patient suspected for malaria. Sample identification numbers were labeled both on blood film slides and RDTs.

Rapid diagnostic test (RDT)

The available RDT used for malaria diagnosis in the region was RDT (SD Bioline). It is a three antigen coated immunochromatographic test. The coated P. falciparum histidine-rich protein II (PfHRP-II) antigen and Plasmodium lactate dehydrogenase (pLDH) antigen are specific to detect P. falciparum and P. vivax, respectively. There are three bands in the kit for P. falciparum, P. vivax and control test line. If three lines appear, it is interpreted as mixed infection. In cases, where the control line did not appear, the results were interpreted as invalid and the test was repeated with a new device. Testing was carried out as per the manufacturer’s instructions.

Microscopic examination

Thick and thin blood films were prepared using frosted end slides and allowed to air-dry and kept in a standard slide box. The dried blood film were fixed with methanol (70%) and kept in a refrigerator between 2–8°C. The slides were transported within 15 days to Bahir Dar Regional Health Research Laboratory Center. Blood films were stained with 10% Giemsa solution for 10–15 min, air dried and examined under light microscope using 1000× magnification. An experienced medical laboratory technologist examined all slides with standard job aids. In addition, an experienced medical parasitologist cross checked all positives slides and 5% of negative slides in the same center.

Data analysis

Data were compiled and entered using Epi-Info version 3.3.6 and checked for its completeness. Then, data were transported and analyzed using the Statistical Package for Social Sciences version 20 software (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp). RDT results were compared with the Gold standard Giemsa light microscopy for specificity, sensitivity, positive and negative predictive values. The inter-readers variability of Giemsa microscopic was assessed by Kappa values. Chi-square tests and logistic regression model with p-value <0.05 were considered for statistical significance.

Ethical considerations

Ethical clearance was obtained from the research and ethical review committee of Amhara National Regional State Health Bureau. All study participants were made aware of the risks involved, benefits and purpose of the study. Written consent was also assured from each participant. Patients with positive test for malaria were treated immediately free of charge as per the protocols of the Ethiopian Federal Ministry of Health.

RESULTS

In this study, out of 1000 suspected malaria cases, 987 were tested for malaria parasite using RDT and 990 using Giemsa light microscopy. Males constituted 53.2% (524) of the study population. The median age was 21 yr (from 1 to 88 yr-old). The majority, i.e. 67.4% (669) of malaria patients were >15 yr and 17.3% (172) were < 5 yr-old.

The overall, positivity rate (PR) of malaria parasites by RDT was 17.1% (95% CI: 14.7–19.4). Using Giemsa blood film microscopy cumulative PR of malaria parasite was 16.5% (95% CI: 14.3–18.9). Statistically significant difference on positivity rate for P. falciparum and P. vivax was observed between RDT and Giemsa microscopy (p = 0.001). For P. falciparum, RDT showed higher PR (11.3%) compared to Giemsa microscopy (6.9%) (p = 0.001). In contrast, RDT revealed lower PR for P. vivax than Giemsa microscopy (p = 0.001) (Table 1). The overall median value of PR of the earlier results was found lower than in this survey. Detail comparisons of PR of 10 health centers between this study and the three months earlier data are depicted in Table 2.

Sensitivity, specificity and predictive values

Considering blood film Giemsa microscopy as “gold standard” method, the RDT (SD BIOLINE) showed a sensitivity of 83.9% and specificity of 96%. The positive predictive values and negative predictive values were 80.4 and 96.8%, respectively. The level of agreement between first reader and second reader for blood film microscopy was moderate (Kappa value = 0.74). The first and second readers disagreed on 15.7 and 8.4% of positive and negative Giemsa light microscopy results (Table 3).
Onsite assessment

Assessment of the laboratory equipments and supplies in 10 health centers showed that 65% of the 20 required items for malaria microscopy diagnosis were present in health centers. All health centers received Giemsa stain from government source/supply. Only six (60%) health centers responded that they participated in the regional external quality assurance scheme. Three (30%) health centers had involved their laboratory unit with the health centers management committee; a group of individuals represented from each unit of a health center to make decision with the health center director. The majority of laboratory professionals (80%) had two year and above experiences for Giemsa microscopy.

Assessment of knowledge and associated factors for malaria

All malaria suspected cases were interviewed for their knowledge, attitude and practice related to malaria prevention and control. Most of the respondents [60.2% (602)] presented themselves at the health facility center seeking for malaria diagnosis and treatment. However, only 48.1% (481) had information about modes of transmission of malaria. Detailed information on knowledge and practices is depicted in Table 4.

The logistic regression model showed that being male (AOR = 1.49, 95% CI = 1.055–2.122), children < 5 yr of age (AOR = 0.323, 95% CI = 0.173–0.602) and patients having fever for >24 h prior to blood test (AOR = 1.57, 95% CI = 1.061–2.325) had statistically significant association with positivity rate for malaria parasites (Table 5).

DISCUSSION

This study revealed that RDT (SD Bioline) had good accuracy with specificity (96%) and negative predictive value (NPV) (96.8%). This conforms to 97.3% of specificity and 94.9% of NPV in Uganda. As expected, the sensitivity and positive predictive values of RDT (SD BIOLINE) in this survey were low. This was lower compared to other studies reported in Nigeria and in Ethiopia. In another study, the RDT (SD Bioline) has shown
47% sensitivity\(^\text{12}\). In this study, RDT showed higher PR for \textit{P. falciparum} compared to blood film examination \((p = 0.001)\). In contrast, it revealed lower detection for \textit{P. vivax} \((p = 0.001)\). However, a previous study in Ethiopia has shown that RDTs had similar detection rate for both \textit{P. falciparum} and \textit{P. vivax}\(^2\).

The sensitivity, positive predictive value, cumulative positivity rate by RDT and microscopy of this study was lower compared to a previous report in Ethiopia\(^\text{11}\). This might be due to the seasonal variability of malaria transmission in the two study periods. During the major transmission season, the chance of getting infected is higher along with higher level and by RDTs at Hes or provide mapparasite load, thus increasing the chance of being detected by RDTs. Likewise, a study conducted in India reported 100% sensitivity for RDT\(^\text{13}\).

The level of agreement between the two diagnostic tests, RDT and microscopy in the study was 0.78 which was in line with the result of a study in Gondar, Ethiopia\(^\text{11}\). Blinded rechecking of blood film slides between first and second readers showed moderate agreement \((\text{Kappa value} = 0.74)\). However, readers showed poor agreement on negative \((15.7\%)\) than positive \((8.3\%)\) microscopic slides. Therefore, setting Giemsa stained blood film microscopy as a gold standard for evaluation of RDT in the absence of PCR for malaria diagnosis is recommendable.

An interesting observation in this study was comparison result of the PR between this survey and the preceding three months positivity data of health centers. The overall PR of three months earlier results/data both by RDT and Giemsa microscopy was lower than that in this survey. However, there were differences in results from different health centers against this survey. The differences are attributable to occurrence of high malaria transmission during the survey and difference in strength of malaria control among districts. Moreover, in the present study, more qualified and experienced medical laboratory technologists/technicians conducted the malaria microscopy diagnosis which might have enhanced the PR.

Onsite assessment showed that 40% of the laboratory technicians were not involved in external quality control schemes. The survey also found that only 20% of health center laboratories gave supportive supervision to health extension workers at health post level regarding malaria diagnosis by RDT. This might be due to loose integration between health centers and health posts or undermining the importance of RDT quality control at health post level.

Assessment of knowledge on malaria transmission showed that 48.1% of participants knew about mosquito bite, as mode of transmission of malaria. The result was similar to another study in Ethiopia, where 47.5% of malaria patients mentioned mosquito bite as a mode of malaria transmission\(^\text{14}\). However, in southern Ethiopia only 15.6% of malaria cases mentioned mosquito bite as transmitter of malaria\(^\text{15}\). Only 22.9% of participants in
this study knew that stagnant water bodies serve as breeding sites for mosquitoes. This is lower than that reported in an earlier study in Ethiopia.

The logistic regression model revealed that males, children <5 yr age and patients having fever for ≥24 h had statistically significant association with malaria parasite positivity. Similarly, other study has also shown significant association of males and age with malaria infection.

The reason for males being more affected by malaria than females could be their frequent travel into low land farm sites, which are mosquito-prone areas. Moreover, males spent more time out of home, mainly during evenings there by more prone to mosquito bites. Having fever for > 24 h might increase the rate of parasite multiplication in the patient, increasing the probability of detection by malaria diagnostic tests from the peripheral blood.

**Limitations**

Molecular techniques could not be used in the study to evaluate the performances of both RDT and Giemsa microscopy. The other limitation was lack of capacity to undertake the study in wide range of districts, including health extension workers at health post level.

**CONCLUSION**

In this survey, RDT (SD BIOLINE) for malaria diagnosis showed good accuracy with excellent specificity and negative predictive values. However, its sensitivity and positive predictive value deemed low. Inter-reader agreement between laboratory technicians on blood film microscopy was moderate. Being male, children under five year old and patients having fever for ≥24 h showed statistically significant association with malaria parasite positivity. Therefore, training of laboratory technicians on RDTs and microscopy for diagnosis of malaria should be in place to monitor quality of diagnostic services. Furthermore, interventions focusing awareness about malaria transmission of the community and laboratory supplies issues should be strengthened at each level in the health system.

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