ORIGINAL ARTICLE

EVALUATION OF THE PERFORMANCE OF THE SD BIOLINE DENGUE RAPID TEST FOR THE DETECTION OF ANTI-DENGUE IGM ANTIBODY IN NORTHWEST ETHIOPIA

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ABSTRACT

Background: *Dengue is a rapidly emerging mosquito-borne viral disease that affects millions of individuals worldwide. A rapid, accurate, and cost-effective test is essential for patient management. This study aimed to evaluate the performance of the rapid SD Bioline dengue test for point-of-care testing in resource-limited countries.*

Method: *This cross-sectional study was conducted from March 2016 to May 2017, in northwest Ethiopia. Blood samples were collected from febrile patients and serum was separated and tested for IgM antibodies against dengue virus by an enzyme-linked immunosorbent assay (ELISA) and the SD Bioline dengue rapid test. The sensitivity, specificity, and the kappa statistic value of the rapid test were calculated against the results of ELISA as the reference test.*

Result: *Six hundred serum samples were tested by anti-dengue virus IgM seropositivity. The sensitivity of the SD Bioline dengue rapid test was found to be 57.9% (95% CI = 48.3%-67.1%), with specificity of 92.8% (95% CI = 90.1%-94.9%), positive predictive value 65.4% (95% CI = 56.9%-72.9%), negative predictive value 90.4% (95% CI = 88.3%-92.1%), and accuracy of 86.2% (95% CI = 83.1%-88.8%). The kappa agreement of the SD Bioline test was 0.53.*

Conclusion: *This study showed that the SD Bioline test has high specificity for the diagnosis of dengue but has low sensitivity and moderate agreement with the reference assay. Hence, a rapid test kit with high sensitivity and better agreement needs to be considered for proper management of dengue patients in countries where a reference diagnostic test is limited.*

Keywords*: Dengue virus infection, SD-Bioline test, ELISA, Performance, Ethiopia*

BACKGROUND

Dengue is the most rapidly spreading viral disease of public health concern throughout tropical and sub-tropical regions worldwide. It is caused by infection with any one of the four dengue virus serotypes (DENV1-4) while recently a fifth serotype (DENV-5) was reported(1).The virus is transmitted to humans through the bite of infected female *Aedes* mosquitoes, mainly by *Aedes aegypti* (2).The disease is endemic in more than 100 countries, with 390 million cases occurring annually(3).Early diagnosis and appropriate clinical management can reduce mortality in severe dengue cases from 20-30% to less than 1%(4, 5).

A 2009 World Health Organization report showed a gradual increase in the global incidence of infection with DENV(2).One of the most important reasons for the increase is believed due to factors such as rapid

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urbanization, which provides breeding sites for the primary dengue vector, *Aedesa egypti*, among expanding human populations. Moreover, the spread of DENV infection is also increased by global travel and international trade, which facilitates the transmission of viremic individuals and mosquito larvae to non-infected areas(6). The global strategic plan is to reduce mortality and morbidity from dengue by at least 50% and 25% respectively(2). Thus, rapid, accurate, and affordable tests that allow early diagnosis of dengue are essential for combating this devastating mosquito-borne disease, by initiating large scale preventive measures for vector control (7).

Infection with DENV can produce a broad spectrum of disease manifestations that range from mild febrile illness to severe, potentially fatal disease. Most of the clinical features of dengue are nonspecific, thus diagnosis is based only on clinical presentation is not reliable. Due to this, laboratory confirmation of clinical diagnosis is necessary. Because some patients progress over a short period from mild to severe disease and sometimes to death, early intervention may be life-saving(2). Many diagnostic methods have been developed for the diagnosis of DENV infection, each with advantages and disadvantages.

These include viral culture, viral RNA detection by reverse transcriptase-polymerase chain reaction (RT-PCR),NS1 antigen detection, and serological tests such as IgM and IgG enzyme-linked immunosorbent assay (ELISA)(8). The isolation of DENV in cell culture provides the most convincing evidence of infection but requires aprolonged testing period and facilities for culture that are not available in most clinical settings in low and middle-income countries (LMICs). Consequently, this method is not commonly used in routine diagnostic laboratories. Detection of viral RNA by RT-PCR provides accurate diagnosis, but requires expensive equipment, reagents, laboratory infrastructure, and well-trained staff; because of this, this approach is infeasible inmost laboratories(9). The viral NS1 detection by ELISA is nowadays used routinely by many laboratories to diagnose patients during the acute phase of dengue disease. However, it too takes several hours to complete, and is not available in most health facilities in LMICs(10). Currently, simple and rapid immunochromatographic tests (ICT) provide alternatives to virus isolation; PCR or ELISA for the diagnosis of dengue are affordable and point-of-care test are commercially available(11).

Now a days dengue is emerging in the different regions of Ethiopia (12-14). However, the major diagnostic methods currently available require infrastructure, laborious procedures, and relatively high cost, which makes them impractical in routine use, particularly in resource-limited countries(9, 10). Dengue rapid ICTs which are simple to use and inexpensive have become available in most developing high-dengue burden countries.

However, their diagnostic performance has been noted to vary in different countries(2,15). Performance variations of these tests and lack of local documented data about the usefulness of dengue diagnostic tests under routine conditions in Ethiopia highlight the need to evaluate the sensitivity and specificity of this commercially available test. Therefore, the present study aimed to evaluate the diagnostic performance of the rapid SD Bioline test compared with the reference test for the diagnosis of dengue in Ethiopia.

METHOD

Study area and period: This study was conducted in Metema and Humera Kahsay Abera hospitals, in northwest Ethiopia, from March 2016 to May 2017.

Study design and population: A cross-sectional study was carried out among 600 dengue presumed patients who fulfilled the WHO clinical criteria of DENV infection upon admission during the study periods. Dengue presumes were defined as patients presenting with fever and two of the following criteria: nausea or vomiting, rash, aches and pains, tourniquet test positive, leucopenia, abdominal pain, persistent vomiting, mucosal bleeding, lethargy, restlessness, liver enlargement > 2 cm and increase in hematocrit (HCT) concurrent with the rapid decrease in platelet count. Symptoms were evaluated according to the 2009 WHO criteria(2).

Specimen collection: Three-five ml of venous blood was collected from each study participant, using red-capped vacutainer tubes with a clot activator centrifuged at 3500 rpm for 5 minutes. The serum was a liquoted into 1.5 ml Eppendorf tubes and stored in -20^{o}C, subsequently transported using dry ice to the Department of Medical Microbiology virology laboratory of the University of Gondar and stored at -70° C until analyzed.

Diagnosis of dengue virus infection: The serum samples were tested by the reference anti-DENV IgM ELISA test (16), and also by the SD Bioline rapid test to detect anti-DENV IgM antibodies. The diagnostic accuracy of anti-DENV IgM ELISA (EUROIMMUN, Lubeck, Germany) according to the manufacturer's report is 100% sensitivity and 98% specificity(17). The commercially available Bioline dengue rapid test (Standard Diagnostics, South Korea) is claimed to have sensitivity of 94.6% and specificity of 96.5% according to the manufacturer's report (18). Both tests were performed and the results were examined and interpreted according to the manufacturer's instructions. Although the SD Bioline test detects both anti-DENV IgM and IgG, the study focused on only IgM results. The dengue-specific IgM test is a useful diagnostic tool in resource-limited countries, particularly after a very short viremia period (i.e., IgM becomes detectable on day 3 to 5 of illness). IgG results were not considered as it indicates past infection(19).

Statistical analysis: Data were entered using SPSS version 20 software. A two by two table was generated in which the result of the reference assay was cross-tabulated with the SD Bioline rapid test and true positives, true negatives, false positives, and false negatives were identified. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were calculated by using medcalc statistical software. Measures of agreement between SD Bioline and ELISA were determined by using kappa statistics, in which agreement was interpreted as follows: < 0.20 slight agreement, 0.21-0.40 fair agreement, 0.41-0.60 moderate agreement, 0.61-0.80 substantial agreement and 0.81-1.00 was considered very good agreement(20).

Ethical consideration: Ethical clearance was obtained from the University of Gondar Ethical Review Committees. After ethical clearance, a letter of agreement and cooperation was written to the selected health care institutions. Written informed participant consent was obtained from the target population after explanation of the purpose and objectives of the study.

RESULT

Out of 600 serum samples tested using the dengue SD Bioline rapid test and IgM ELISA, 101 (16.8%) serum samples were positive for anti-DENV IgM by SD Bioline test while 114 (19%) serum samples were positive for the anti-DENV IgM by ELISA. When the SD Bioline anti-DENV IgM test results were compared with the reference ELISA anti-DENV IgM test, 66 were true positives, 451 were true negatives, while there were 48 false negatives and 35 false positive results in the SD Bioline test (Table 1). Detailed socio-demographic and seroprevalence data were published in our previous study(14).

Table 1: Two by two table of the anti-DENV IgM test of SD-Bioline against reference ELISA in northwest Ethiopia, from March 2016 to May 2017.

Anti-DENV IgM SD Bioline rapid test	Anti-DENV IgM ELISA test		Total
	Positive	Negative	
Positive	66	35	101
Negative	48	451	499
Total	114	486	600

Compared with ELISA, the dengue SD Bioline IgM test showed a sensitivity of 57.9% (95% CI = 48.3%-67.1%), specificity 92.8% (95% CI = 90.1%-94.9%), positive predictive value 65.4% (95% CI = 56.9%-72.9%), negative predictive value 90.4% (95% CI = 88.3%-92.1%), and accuracy of 86.2% (95% CI = 83.1%-88.8%). The kappa value of the test was 0.53 (Table 2).

Table 2: The diagnostic performance of the anti-DENV IgM test of SD-Bioline against reference ELISA in northwest Ethiopia, from March 2016 to May 2017

Indices	SD Bioline IgM test
Sensitivity% (95% CI)	57.9 (48.3-67.1)
Specificity% (95% CI)	92.8(90.1-94.9)
Positive predictive value% (95% CI)	65.4 (56.9-72.9)
Negative predictive value% (95% CI)	90.4 (88.3-92.1)
Accuracy% (95% CI)	86.2 (83.1-88.8)
Kappa value	0.53

DISCUSSION

This is the first study that provides evidence on the diagnostic performance of the dengue rapid SD-Bioline test in Ethiopia. A rapid and accurate method for the diagnosis of dengue is essential for clinicians to provide appropriate clinical management for patients and also to closely follow disease-specific warning symptoms associated with severe disease. Although several commercially available dengue rapid diagnostic tests are used, their sensitivity and specificity have shown to have large variations in different countries(2, 15).

In this study, the sensitivity of the SD Bioline test for the detection of anti-DENV IgM antibody was 57.9% and its specificity was 92.8%. This finding is in agreement with an earlier study, which showed a test sensitivity of 60.9% and specificity of 90%(21). However, lower sensitivity (32.7%) and specificity (86.2%) was reported in Cambodia(22);and higher sensitivity (70%) but lower specificity (76.5%) was reported in Nepal(23). Moreover, highersensitivity (79%) and specificity (95%) was also reported in

Thailand(9). A rapid dengue diagnostic SD Bioline test has thus shown to have large variations in sensitivity and specificity between different studies. This heterogeneity of test sensitivity and specificity might be due to differences in sample collection after the onset of the disease(9). Other confounding factors for this variation in study results may include the prevalence of other febrile endemic diseases.

The results of the present study showed that the SD Bioline test has PPV of 65.4% and NPV of 90.4% compared with reference assay. The high NPV suggests that this test may reliably identify those who do not have dengue while the low PPV indicates that the test may not reliably indicate those who do have the disease. The sensitivity (57.9%) and specificity (92.8%) of the SD Bioline rapid test for the detection of anti-DENV IgM antibody in the present study were lower as compared to the manufacturer's performance characteristic sensitivity (94.6%) and specificity (96.5%) (18). The lower sensitivity in this study suggest that it is unsuitable for distinguishing DENV infection from other infections and it does not perform as well as reported by the manufacturer of the test.

As dengue is increasing globally, it is necessary to have a rapid diagnostic test with good diagnostic accuracy in resource-limited countries. Rapid dengue diagnostic ICTs are simple point-of-care tools to speed up the differential diagnosis of DENV infection and its clinical management(19). The SD Bioline test has some limitations; Viral RNA PCR and viral isolation are not done because of the limitations of facilities. Hence, the study did not identify dengue virus serotype and the issues of potential antibody cross-reactivity with the other aviviruses. The acute versus convalescent serum sample to demonstrate dengue sero conversion was not done due to the nature of the cross-sectional study in which a single serum sample was used. Despite these limitations, this study could provide the first baseline information regarding the test performance of the SD Bioline rapid test for detection of anti-dengue IgM antibodies.

In conclusion, the findings of this study showed that the SD Bioline rapid test for the detection of dengue specific IgM antibodies had sensitivity and specificity of 57.9% and 92.8%, respectively. In countries like Ethiopia where reference tests are limited, future studies should focus on evaluating a rapid test with high sensitivity and a better agreement needs to be considered for proper management of dengue.

Conflicts of interest: The authors declare that there is no conflict of interest regarding the publication of this article.

Data availability: The data used to support the findings of this research are available from the corresponding author upon reasonable request.

Funding: This study was funded by the University of Gondar and Armauer Hansen Research Institute.

Author's contributions: GF: participated in the conception and design of the study, data collection, laboratory analysis, data analysis and interpretations of the findings, drafted the manuscript and wrote the paper. MT and EA: participated in the conception and design of the study, data analysis and interpretations of the findings. YW: participated in the data collection, data analysis, and interpretations of the findings. EG, RH, AA: participated in the conception and design of the study, interpretations of the findings, BT: participated in the conception and design of

the study, supervised during data collection and laboratory analyses, data analysis and interpretations of the findings. All authors reviewed and approved the final manuscript.

ACKNOWLEDGMENT

We would like to thank the University of Gondar and Armauer Hansen Research Institute for providing support. The role of the Tropical and Infectious Diseases Research Center at the University of Gondar was immense contributions in the process. We would also like to thank all the data collectors in the Metema and Humera Kahsay Abera hospitals and also all the study participants without their participation it would have not been possible to do this research.

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