ISSN: 0975-3583, 0976-2833 VOL15, ISSUE7, 2024

Original Research Article

Title: Assessing the effectiveness of popular reference laboratory tests for diagnosing acute dengue: A Meta-analysis and systematic review of RT-PCR, NS1 ELISA, & IgM ELISA

Authors: Dr. Juhi Sisodia[1] (Assistant Professor) & Dr. Sanjay Kumar Singh[2] (Assistant Professor)

Dept. of Microbiology, Autonomous State Medical College, Sultanpur[1]

Dept. of Pathology, Autonomous State Medical College, Sultanpur[2]

Corresponding Author: Dr. Sanjay Kumar Singh

Abstract

Background & Methods: The aim of the study is to assess the effectiveness of popular reference laboratory tests for diagnosing acute dengue: a meta-analysis & systematic review of RT-PCR, NS1 ELISA, & IgM ELISA. Dengue is a widely spread arboviral disease in tropical & subtropical regions of the world. Dengue fever presents clinical characteristics similar to other febrile illness. Thus laboratory diagnosis is important for adequate management of the disease.

Results: Three tests of interest were able to analyze data from articles over a variety of time periods. The combined sensitivities of IgM ELISA (1–7 days), RT-PCR (0–4 days after symptom start), and NS1 ELISA (0–4 days) were 71% (57–84), 90% (68–98), and 95% (95% credible interval 77–99), respectively. Specificity was estimated to be 89% (60–98), 93% (71–99), and 91% (82–95) in the corresponding pooled estimations. Similar results were found in a subanalysis of only studies with a low probability of bias.

Conclusion: Our review indicates that because of its low diagnostic accuracy during the initial days of symptom onset, IgM ELISA should not be performed as a stand-alone test at this time. Furthermore, we discover that NS1 ELISA & RT-PCR have comparable diagnostic accuracy. Our estimations serve as a foundation for upcoming Bayesian & conventional analysis methods that evaluate the accuracy of dengue tests intended for near-patient use, such as in the assessment of innovative RDTs & diagnostic algorithms. Particularly with regard to the usefulness of NS1 ELISA, these findings have consequences for global health & surveillance policy, as well as for the development, assessment, & regulation of dengue diagnostic tests.

Keywords: diagnosing, acute, dengue, RT-PCR, NS1 ELISA, & IgM ELISA.

Study Design: Systemic Review.

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE7, 2024

Introduction

Dengue is a widespread disease spread by mosquitoes worldwide. In tropical & subtropical regions, over 2.5 billion people are susceptible to infection [1]. Every year, around 50 million illnesses happen worldwide [2]. Since notification is ineffective & laboratory assays are not always available in less developed nations, the true prevalence of dengue is most likely higher. The most common way that viruses are spread is by mosquito bites from Aedes aegypti & Aedes albopictus. DENV-1, DENV-2, DENV-3, & DENV-4 are the four virus serotypes that have been identified [3]. Clinical manifestations of dengue range from an undifferentiated fever to the severe hemorrhagic form, known as dengue hemorrhagic fever (DHF), which can cause shock & even death.

Rainfall affects the density of dengue vectors [6]. As a result, dengue fever incidence in Brazil increases annually in the summer following the rainy season. Additionally, the frequency fluctuates greatly from year to year in the various parts of the nation. The Brazilian Ministry of Health's Department of Health Surveillance reported incidences of 205.5, 530.3, & 400.5 per 100,000 people, respectively, between 2009 & 2011 [7]. Dengue is undoubtedly a significant health issue in Brazil. Therefore, accurate & timely diagnosis is crucial for effective illness management. Unspecific symptoms are the hallmark of dengue fever, & the illness typically presents clinically similarly to other viral & febrile illnesses. For the final diagnosis of dengue, clinical criteria are therefore not the best option [8].

Acute dengue infection can be identified using a variety of diagnostic techniques, such as virus isolation, RT-PCR & real-time PCR, viral genome sequencing, viral antigen identification, & serologic techniques. Serology is a widely utilized technique in public health services in Brazil. Although RT-PCR & NS1 antigen identification by ELISA are sensitive techniques for early dengue virus infection detection [9], Brazilian public health services do not make extensive use of them. Furthermore, laboratory diagnosis is not always available in regions with high incidence rates during epidemic seasons. To identify potential dengue infections in the latter situation, doctors must rely on clinical & epidemiological criteria, which may result in incorrect diagnoses & the failure to identify other viral viruses that are significant for public health [10]. Primary care services have recently begun using lateral flow immunochromatographic tests for the identification of the NS1 antigen. These tests are simple to administer, inexpensive when used for large-scale surveys, & straightforward to distribute to distant clinical institutions for major medical centers in big nations like Brazil. Our objective was to ascertain their relative utility in an actual clinical epidemic scenario.

Material & Methods

It is a systemic review conducted at Department of Microbiology. The consent was taken from each patients during enrolment in this study. The inclusion & exclusion criteria were followed as mentioned below:

Inclusion criteria

1. Age: All age groups.

2. Clinically suspected cases of dengue fever visiting to O.P.D. & I.P.D.

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE7, 2024

Exclusion criteria

1. All previously diagnosed cases of dengue infection & febrile cases with other proven aetiology (malaria, typhoid, other febrile illnesses etc.).

Blood samples (3-4 ml) were collected under all aseptic precautions from patients who fulfilled the inclusion criteria. The samples (Serum/Plasma) separated by centrifugation for serological & molecular diagnosis.

Patients signs & symptoms

The history of most common clinical signs & symptoms like fever, arthralgia, myalgia, retro-orbital pain, headache, rashes, epistaxis & melena, & vomiting were taken from all the patients.

NS1 antigen ELISA

Non-structural (NS1) antigen ELISA was performed using QualisaTM Dengue NS1 kit as per manufacturer protocol.

Dengue IgM antibody capture (MAC) ELISA

Dengue antibody detection was done by MAC IgM ELISA kit,

RNA extraction & real time reverse transcriptase PCR.

Real time reverse transcriptase PCR for serotyping was performed by Geno Sen's dengue 1-4 Kit (Gemone Diagnostic Pvt Ltd, HP, India) in 7500 fast Dx real-time PCR instrument.

Unique results were imported into a systematic review software, CADIMA,12 for abstract screening. Papers were selected from over participants, were in English, were published in a peer-reviewed journal, & presented results for at least one TOI (NS1, IgG, or IgM ELISA, RT-PCR, or VNT) against a comparator using the same human serum sample. For serology (ie, IgGor IgMELISA), paired samples were used if the first sample was tested against the comparator.

Result

IgM ELISA included 67 828 individuals & had the most investigations (n=107). Five of the six WHO regions were represented in the included studies. Of these, 124 (91%) were from India, & the remaining half (137 [49%] of 282) were from South-East Asia. Eight [13%] of 60 for NS1 ELISA, 11 [14%] of 78 for RT-PCR, & 18 [17%] of 107 for IgM ELISA were among the 37 (15%) of 245 studies that published findings separately for adults & children. Comparably, results for inpatients & outpatients were published separately in 25 (14%) of 245 investigations (12 [20%] of 60 for NS1 ELISA, 12 [15%] of 78 for RT-PCR, & 21 [20%] of 107 for IgM ELISA).

Variability in the timing of diagnostic tests following fever start was revealed by the extracted data. Out of 245 papers, 101 (41%) reported that acute dengue testing was done on symptomatic people without mentioning DPO. This percentage was comparable for all TOIs (NS1 ELISA: 22 [37%] of 60, RT-PCR: 37 [47%] of 78, & IgM ELISA: 42 [39%] of 107).

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE7, 2024

Our analysis's estimated sensitivity & specificity for every TOI. Appendix 1 (pp. 45–53) presents individual study estimates for sensitivity & specificity for each TOI in each DPO subgroup, illustrating the variability in comparator tests & the heterogeneity of results.

The sensitivity of RT-PCR & NS1 ELISA declined with time after symptom onset, according to a comparison of findings from 0–4 DPO, 1–7 DPO, & the whole acute symptomatic group. At 0–4 & 0–7 DPO, respectively, RT-PCR sensitivity dropped from 90% (95% CrI 68–98) to 86% (68–96).

Similarly, from 95% (77–99) to 85% (69–93), NS1 ELISA dropped. Overall, RT-PCR's sensitivity & specificity point estimations were still above 85% & 90%, respectively. Similarly, NS1 ELISA point estimates for specificity & sensitivity were above 90% & above 85%, respectively, regardless of the DPO subgroup. Appendix 2 (pp. 1–7) has forest plots that display all investigations for each TOI for each time period. There was a noticeable difference in IgM ELISA sensitivity between the subgroup analyses of findings reported at 1–7 DPO & 0–4 DPO, with 71% (95% CrI 57–84) & 17% (3–51), respectively. Sensitivity & specificity estimates for the IgM ELISA primary analysis model (1–7 DPO) were lower than those for the model that included all acute symptomatic data, at 62% (45–75) & 85% (76–91%), respectively. IgM ELISA sensitivity was 82% (49–96) at 5–14 DPO compared to 82% (45–96) at 1–7 DPO. For all cases of acute dengue, the PanBio Capture IgM ELISA has a sensitivity of 86% (68–94) & a specificity of 87% (72–95).

Wider CrI for the forecast estimates relative to the pooled estimates showed heterogeneity across all meta-analyses. The primary analytic model for all TOIs had the narrowest CrI for the anticipated values, whereas the all acute symptomatic model had the greatest CrI.

Discussion

The effectiveness of real-time PCR in an actual epidemic setting has not been well-documented. One of the worst epidemic peaks ever recorded in Belo Horizonte occurred during the February–May 2010 research period in the study region [11]. We discussed dengue early diagnostic options by evaluating the effectiveness of lateral flow tests for NS1 detection, NS1 ELISA, & real-time PCR. The sensitivity of the real-time PCR was comparable to that of the NS1 ELISA. Because they were easier to use & less costly than other techniques, lateral flow tests for NS1 detection were the most practical approaches for dengue early diagnosis.

For the clinical therapy of dengue, early diagnosis is crucial & could avoid poor results [12]. The effectiveness of the various dengue diagnosis techniques varies depending on the stage of the illness. The sensitivity of dengue diagnostic techniques such RT-PCR & real-time PCR, virus isolation, or NS1 detection is higher in the early stages of sickness because dengue virus & dengue viral products are found in serum during this time. However, when larger levels of anti-dengue immunoglobulins are found in blood during the convalescent period (about 5–6

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE7, 2024

days after the onset of sickness), serologic techniques such as anti-dengue IgM perform better for diagnosing dengue [13]. Because they feel better, many patients do not return to the site of care for blood collection, which is a drawback of this approach. Early diagnosis would be beneficial for an efficient treatment approach because dengue hemorrhagic syndrome emerges as fever lowers, which happens about the same time.

The sensitivity of the real-time PCR for diagnosing dengue was good. When compared to NS1 ELISA alone & serological methods combined, the method's overall performance was satisfactory, & it correlated with the serological diagnosis results employed in this investigation for case definition (positive NS1 ELISA and/or positive anti-dengue IgM ELISA). These results are consistent with earlier research that detailed alternative real-time PCR procedures [14].

The unspecific amplification of SLEV sequences by the real-time PCR employed here obviously restricts its generalizability. In the context of epidemic scenarios like the one that occurred during the research period, it might be helpful. Interestingly, other research have not investigated the specificity against other flaviviruses, although using a comparable design (i.e., the same or a similar pair of primers). Although the virus can occasionally cause encephalitis, SLEV infection in people is typically accompanied by a subclinical or moderate febrile illness. In North America, where erratic epidemics have occurred in recent decades, this is epidemiologically relevant. In the state of São Paulo in 2007, a significant DENV-3 outbreak coincided with an outbreak of SLEV [15].

Therefore, for diagnostic purposes based on real-time PCR, in addition to plain nucleic acid amplification data, analysis of the melting curves of the amplified products, which displayed different profiles for Dengue & SLEV, should be taken into consideration. This is because mild cases of SLEV exhibit an acute febrile illness that is indistinguishable from dengue fever, especially during the acute phase of infection. We further recommend that a particular qRT-PCR for Saint Louis be carried out in a rare clinical setting (such as a suspected case of viral encephalitis). Therefore, individuals who exhibit unusual clinical symptoms should not be treated with our technology. Given the intricacy of real-time PCR & the unique problem of our method's specificity, lateral flow tests were a more practical & highly accurate way to identify NS1 for dengue early diagnosis.

However, compared to original DENV infection, IgM titres increase less in response to secondary infection, which limits the test's usefulness, especially in dengue-endemic areas. Subanalysis was not possible since few of the papers in this review differentiated between primary & secondary infections. The accuracy of IgM ELISA can be improved by utilizing paired samples, interpreting the results as a ratio with IgG ELISA, or combining a single measurement with other diagnostic techniques as part of a diagnostic algorithm, even though it is frequently used as a single test in the acute phase.

Priority should be given to meta-analyses to determine the diagnosis accuracy of dengue algorithms. Commercial NS1 & IgM ELISA assays for acute dengue infection have been shown to perform differently in terms of diagnosis. A sub-analysis of the PanBio Capture IgM ELISA within the all acute symptomatic group revealed higher sensitivity (86% [95%

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE7, 2024

CrI 68–94]) compared with all brands (62% [45–75]), despite the fact that there was not enough data to conduct additional analyses for each distinct commercial test at each timeframe. However, CrIs overlapped. Our results emphasize the necessity of comparing commercial ELISAs with well-characterized serum panels in order to inform assay selection [16]. The thorough & reliable search approach used in this analysis, which covered five reference laboratory tests for dengue diagnosis across 11 bibliographic databases & found research from 43 countries with a variety of comparators, is one of its strong points. Additionally, the technique followed PRISMA & QUADAS-2 principles. Our Bayesian analysis did not make the assumption that any comparator was flawless. To the best of our knowledge, this method has never been utilized on a dataset this size for our TOIs, despite having been used in the past for quick tests for dengue.

Conclusion

Our review indicates that because of its low diagnostic accuracy during the initial days of symptom onset, IgM ELISA should not be performed as a stand-alone test at this time. Furthermore, we discover that NS1 ELISA & RT-PCR have comparable diagnostic accuracy. Our estimations serve as a foundation for upcoming Bayesian & conventional analysis methods that evaluate the accuracy of dengue tests intended for near-patient use, such as in the assessment of innovative RDTs & diagnostic algorithms. Particularly with regard to the usefulness of NS1 ELISA, these findings have consequences for global health & surveillance policy, as well as for the development, assessment, & regulation of dengue diagnostic tests.

References

1. M.G. Guzman, S.B. Halstead, H. Artsob, P. Buchy, J. Farrar, D.J. Gubler, et al. Dengue: a continuing global threat Nat Rev Microbiol, 8 (December (12 Suppl.)) (2010), pp. S7-S16
2. S.O. De Paula, B.A. Fonseca Dengue: a review of the laboratory tests a clinician must know to achieve a correct diagnosis Braz J Infect Dis, 8 (December (6)) (2004), pp. 390-398
3. R. Lima Mda, R.M. Nogueira, H.G. Schatzmayr, F.B. dos Santos Comparison of three commercially available dengue NS1 antigen capture assays for acute diagnosis of dengue in Brazil PLoS Negl Trop Dis, 4 (7) (2010), p. e738
4. A.C. Terzian, A. Mondini, R.V. Bronzoni, B.P. Drumond, B.P. Ferro, E.M. Cabrera, et al. Detection of Saint Louis encephalitis virus in Dengue-suspected cases during a dengue 3 outbreak Vector Borne Zoonotic Dis, 11 (March (3)) (2011), pp. 291-300
5. S.O. De Paula, C. Nunes, R. Matos, Z.M. de Oliveira, D.M. Lima, B.A. da Fonseca Comparison of techniques for extracting viral RNA from isolation-negative serum for dengue diagnosis by the polymerase chain reaction J Virol Methods, 98 (November (2)) (2001), pp. 119-125
6. L.J. Chien, T.L. Liao, P.Y. Shu, J.H. Huang, D.J. Gubler, G.J. Chang Development of real-time reverse transcriptase PCR assays to detect & serotype dengue viruses J Clin Microbiol, 44 (April (4)) (2006), pp. 1295-1304
7. R.S. Lanciotti, C.H. Calisher, D.J. Gubler, G.J. Chang, A.V. Vorndam Rapid detection & typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction J Clin Microbiol, 30 (3) (1992), pp. 545-551

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE7, 2024

8. R.M. Nogueira, J.M. de Araujo, H.G. Schatzmayr Dengue viruses in Brazil, 1986–2006 Rev Panam Salud Publica, 22 (November (5)) (2007), pp. 358-363
9. I.M. Rocco, V.R. Silveira, A.Y. Maeda, S.J. Silva, C. Spenassatto, I. Bisordi, et al. First isolation of Dengue 4 in the state of Sao Paulo, Brazil, 2011 Rev Inst Med Trop Sao Paulo, 54 (January–February (1)) (2012), pp. 49-51
10. J.A. Potts, A.L. Rothman Clinical & laboratory features that distinguish dengue from other febrile illnesses in endemic populations Trop Med Int Health, 13 (November (11)) (2008), pp. 1328-1340
11. S. Ranjit, N. Kissoon Dengue hemorrhagic fever & shock syndromes Pediatr Crit Care Med, 12 (January (1)) (2011), pp. 90-100
12. R.W. Peeling, H. Artsob, J.L. Pelegrino, P. Buchy, M.J. Cardosa, S. Devi, et al. Evaluation of diagnostic tests: dengue Nat Rev Microbiol, 8 (December (12 Suppl.)) (2010), pp. S30-S38
13. L.A. Castro-Jorge, P.R. Machado, C.A. Favero, M.C. Borges, L.M. Passos, R.M. de Oliveira, et al. Clinical evaluation of the NS1 antigen-capture ELISA for early diagnosis of dengue virus infection in Brazil J Med Virol, 82 (August (8)) (2010), pp. 1400-1405
14. B.L. Innis, A. Nisalak, S. Nimmannitya, S. Kusalerdchariya, V. Chongswasdi, S. Suntayakorn, et al. An enzyme-linked immunosorbent assay to characterize dengue infections where dengue & Japanese encephalitis co-circulate Am J Trop Med Hyg, 40 (April (4)) (1989), pp. 418-427
15. H.W. Dos Santos, T.R. Poloni, K.P. Souza, V.D. Muller, F. Tremeschin, L.C. Nali, et al. A simple one-step real-time RT-PCR for diagnosis of dengue virus infection J Med Virol, 80 (August (8)) (2008), pp. 1426-1433
16. B.W. Johnson, B.J. Russell, R.S. Lanciotti Serotype-specific detection of dengue viruses in a fourplex real-time reverse transcriptase PCR assay J Clin Microbiol, 43 (October (10)) (2005), pp. 4977-4983