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Original Research Article

Comparison of rapid dengue NS1, qualitative ELISA, and quantitative ELISA result

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ABSTRACT

Background: Non-structural glycoprotein-1 (NS1) is a useful biomarker for early diagnosis of dengue fever. NS-1 antigen ELISA can be used for the early diagnosis of dengue fever in the acute stage. Quantitative methods are better for epidemic settings due to high false negative rates in qualitative ELISA.
Methods: The study was initiated after approval from the institutional ethics council (IEC/DISS/17118). Study examined 280 patients with dengue symptoms who presented to the hospital's OPDs and IPDs. Patients were tested using qualitative ELISA, and those with Leptospira antibody, malaria, or Chikungunya IgM antibody were excluded. Age, gender, symptoms, comorbidities, total leucocyte count, platelet count, and risk category were all patient-related parameters. Patient-related parameters were recorded, and data was collected using Microsoft excel and analysed statistically.
Results: Most patients aged 2-40 with male predominance had fever, chills, and body aches, 243 (86.8%) tested positive for ELISA NS1. Quantitative ELISA test showed a statistically significant correlation with rapid antigen NS1 result (p=0.015). Its AUC was 0.883 (p=0.0001), and its cut-off was (>109.1) with 96.9% sensitivity and 13.64% specificity. The AUC of quantitative ELISA NS1 against qualitative ELISA NS1 was 0.853 which was statistically significant (p<0.0001). At the cut-off >74.34, the test's sensitivity was 92.59% and specificity was 75.68%.
Conclusions: Qualitative ELISA NS1 test is better than rapid antigen test for screening due to its higher specificity and similar sensitivity.

Keywords: Dengue, NS-1 dengue, RATs, Qualitative ELISA, Quantitative ELISA, PPV, NPV, Sensitivity, Specificity

INTRODUCTION

The antigen nonstructural-1 (NS-1) is important for DENV replication in the host cell. The antigen is synthesized and released into the bloodstream of infected patients, making it an important biomarker for diagnosing flavivirus infection at an early stage.[1-3] NS1 assays are particularly useful in clinical settings because they can detect the DENV's acute phase, and NS1 lasts longer in the blood than viremia.[4-8] Quick detection techniques are usually lateral flow-based rapid assays, and antigen-capture ELISAs are utilised in laboratory-based testing.[9] The NS1-based assays, according to the CDC, have similar results to molecular tests in the first week of infection, therefore showing a promising potential as a diagnostic tool.[10]

The use of qualitative ELISA confers several advantages, including a simple protocol and high sensitivity and specificity, owing to its dependence on an antigen-antibody reaction. Qualitative ELISA represents an efficient method capable of analysing samples concurrently without the need for intricate pre-treatments.

Moreover, it is generally regarded as a safe and eco-friendly technique as it does not necessitate the use of radioactive substances or substantial amounts of organic solvents.[11]

In diagnostic laboratories, the NS1 antigen ELISA can be used to diagnose dengue fever in acute stage. Test could potentially be useful in epidemic settings, allowing for early patient screening therefore, limiting disease spread.

This study was conducted to aid in the speedy detection of this severe illness by quantitative approaches that were missed by qualitative methods for early diagnosis, disease surveillance, and timely management to save lives.

METHODS

The study was initiated after getting an approval from the institutional ethics council (IEC/DISS/17118). The study was carried out over the course of a year and a half. Dengue symptoms were examined in patients who presented to the hospital including OPD and IPD. A sample size of 280 symptomatic individuals were selected and subjected to qualitative ELISA before being further evaluated. Blood samples were collected from suspected dengue patients after obtaining informed consent. Patients who tested positive for Leptospira IgM antibody, malaria antigen/Chikungunya IgM antibody were excluded from participating in study. Age, gender, symptoms, comorbidities, total leucocyte count, platelet count, and risk category, all patient-related parameters.

The case record form (CRF) was used to acquire a full clinical history as well as the required test data for comparison. Data was collected using Microsoft Excel and statistical analysis was performed.

Study duration

The study conducted from June 2018 to Jan 2019 (course of a year and half).

Study site

Study carried out at Lokmanya Tilak municipal medical college and general hospital, Sion, Mumbai

Sampling technique

Convenient sampling from specimens collected from patients, both outpatient and inpatient department in a tertiary care hospital for first time.

Eligibility criteria

Inclusion criteria

All patients with dengue NS1 antigen positive in rapid test and all patients with dengue NS1 antigen positive by qualitative ELISA were included in study.

Exclusion criteria

Patient is Leptospira IgM antibody positive. Patient is Malaria antigen positive and patient is positive for Chikungunya IgM antibody were excluded.

RESULTS

Most of the patients were in the age group 21-40 years.

The mean age of the patients was 28.01±12.59 years (range: 2 to 78 years). Male predominance (68.21%) was observed.

Table 1: Distribution according to age, sex.

Demographic data		N	Percentage (%)
Age (in years)	≤20	57	20.4
	21-40	182	65.0
	41-60	39	13.9
	>60	2	0.7
	Total	280	100.0
Sex	Female	89	31.8
	Male	191	68.2
	Total	280	100.0

Table 2: Distribution according to presenting symptoms.

Presenting symptoms	N	Percentage (%)
Fever	280	100.0
Chills	268	95.7
Body ache	267	95.4
Headache	1	0.4
Irritability	1	0.4
Stomach-ache	1	0.4
Vomiting	1	0.4

Fever, chills, and body ache were the most common presenting symptoms.

Table 3: Distribution according to qualitative ELISA NS1 result.

ELISA NS1 result	N	Percentage (%)
Negative	37	13.2
Positive	243	86.8
Total	280	100.0

Most of the patients 243 (86.8%) were found to be ELISA NS1 positive by qualitative method.

A statistically significant association was observed between the rapid antigen NS1 result and quantitative ELISA test result (p=0.015).

The above shows the receiver's operating characteristics of quantitative ELISA NS1 for the prediction of COVID-19 against rapid antigen test. Positive groups (n=269) were identified as 'positive' on rapid antigen tests.

The AUC of quantitative ELISA against rapid antigen test was 0.883, which was found to be statistically significant (p<0.0001). The cut-off of quantitative ELISA was (>109.1). At this cut-off, the sensitivity and specificity of 96.90% and 13.64% respectively.

The sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of rapid antigen NS1 test against quantitative ELISA test result were evaluated.

Sensitivity:96.90%, specificity:13.64%, positive predictive value:92.94%, negative predictive value:27.27%, diagnostic accuracy:90.36%.

The rapid antigen NS1 test had specificity (13.64%) with sensitivity (96.90%) and negative predictive value (27.27%) and positive predictive value (92.94%) in the diagnosis of dengue against quantitative ELISA.

Table 4: Association between quantitative ELISA and rapid antigen NS1 result.

Variables		Quantitative ELISA result, n (%)		Total, n (%)
		Negative	Positive	
Rapid antigen NS1 result	Negative	3 (27.3)	8 (72.7)	11 (100)
	Positive	19 (7.1)	250 (92.9)	269 (100)
	Total	22 (7.9)	258 (92.1)	280 (100)

*Pearson chi-square test applied. † Chi-square value=5.962, df=1, p=0.015, significant

Table 5: ROC of quantitative ELISA results in the prediction of COVID-19 against rapid antigen test.

Variables	Quantitative ELISA NS1
Classification variables	Rapid antigen
Sample size	280
Positive group a	269 (96.07%)
Negative group b	11 (3.93%)
Area under the ROC curve (AUC)	
Area under the ROC curve (AUC)	0.883
Standard error	0.0402
95% confidence interval c	0.840 to 0.918
z statistic	9.525
Significance level p (Area=0.5)	<0.0001
Youden index	
Youden index J	0.7455
Associated criterion	>109.1
Sensitivity	83.64
Specificity	90.91

aRapid antigen=1, b rapid antigen=0, cBinomial exact

Table 6: Sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of rapid antigen NS1 test against quantitative ELISA test result.

Variables		Quantitative ELISA result		Total
		Negative	Positive	
Rapid antigen NS1 result	Negative	3 (TN)	8 (FN)	11
	Positive	19 (FP)	250 (TP)	269
	Total	22	258	280

*TN: True Negative, FN: False Negative, FP: False Positive, TP: True Positive.

Table 7: ROC of quantitative ELISA results in the prediction of COVID-19 against qualitative ELISA NS1 test.

Variables	Quantitative ELISA NS1
Classification variables	Qualitative ELISA NS1
Sample size	280
Positive group a	243 (86.79%)
Negative group b	37 (13.21%)

Continued.

Variables	Quantitative ELISA NS1
Area under the ROC curve (AUC)	
Area under the ROC curve (AUC)	0.853
Standard error	0.0390
95% Confidence interval c	0.807 to 0.893
z statistic	9.056
Significance level p (Area=0.5)	<0.0001
Youden index	
Youden index J	0.6827
Associated criterion	>74.34
Sensitivity	92.59
Specificity	75.68

a Qualitative ELISA NS1=1, b qualitative ELISA NS1=0, c Binomial exact.

Table 8: Sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of quantitative ELISA NS1 test (ROC Cut-off) against qualitative ELISA NS1 test result.

Variables		Qualitative ELISA NS1 result		Total
		Negative	Positive	
Quantitative ELISA NS1 result (ROC cut-off)	Negative	28 (TN)	18 (FN)	46
	Positive	9 (FP)	225 (TP)	234

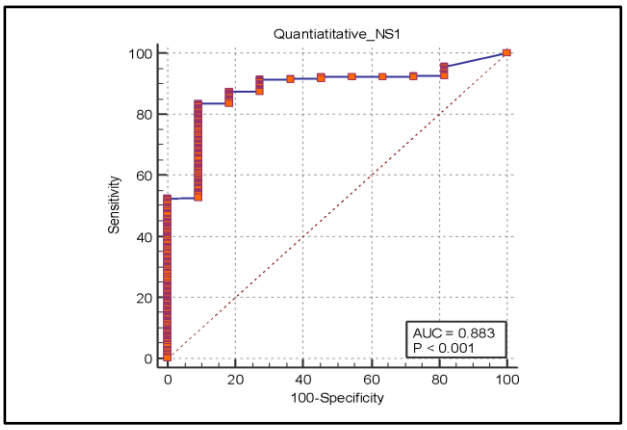

Figure 1: ROC of quantitative ELISA results in the prediction of COVID-19 against rapid antigen test.

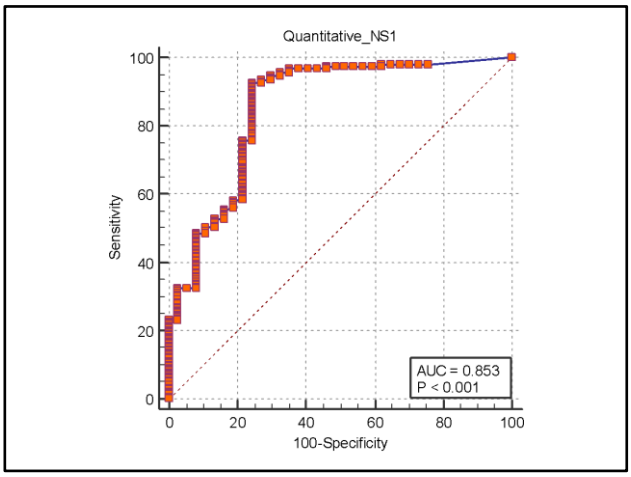

Figure 2: ROC of quantitative ELISA results in the prediction of COVID-19 against qualitative ELISA NS1 test.

The above shows the receiver's operating characteristics of quantitative ELISA NS1 for the prediction of COVID-19 against qualitative ELISA NS1.

Positive groups (N=243) were identified as 'Positive' on qualitative ELISA NS1.

The AUC of quantitative ELISA NS1 against qualitative ELISA NS1 was 0.853, which was statistically significant (p<0.0001). The cut-off of quantitative ELISA NS1 was >74.34. At this cut-off, the sensitivity and specificity of the test were 92.59% and 75.68%.

The ROC cut-off was >74.34. Patients with quantitative ELISA NS1 >74.34 were considered 'Positive' and patients with quantitative ELISA NS1 ≤74.34 were considered 'negative'.

The sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of quantitative NS1 test (ROC Cut-off) against qualitative ELISA NS1 result were evaluated.

Sensitivity:92.59%, specificity:75.68%, positive predictive value:96.15%, negative predictive value:60.87%, diagnostic accuracy:90.36%

The quantitative NS1 (ROC cut-off) test had sensitivity (92.59%), specificity (75.68%), positive predictive value (96.15%), and negative predictive value (60.87%) against qualitative NS1 test.

DISCUSSION

The glycoprotein NS-1 is a highly conserved glycoprotein that is required for dengue virus (DV) viability and is generated by the virus in both membrane-

associated and secretory forms. The presence of significant amounts of NS1 antigen (NS1 Ag) in the sera of DV infected patients during the early clinical phase of the disease has been demonstrated using enzyme-linked immunosorbent assays (ELISA). The measurement of secretory NS1 protein is a novel method for detecting acute DV infection.[12]

In a study by Kulkarni et al Panbio-NS1/IgM-ELISAs identified dengue in 38.6% of patients.[13] All of the tests were less sensitive for IgM detection when compared to Panbio-ELISA, while JM-RDT was less sensitive for NS1. The sensitivity of all tests for combined diagnosis (both markers) was low (55.7-76.6%). Panbio-ELISA was 84% sensitive for NS1, 86% specific for IgM, and 87% specific for combined diagnosis, according to Bayesian latent class analysis (BLCA). As a result, BLCA significantly improved the performance of the other tests; however, the sensitivity of both RDTs for IgM detection remained unacceptable. All four DENV serotypes were detected by NS1 ELISAs and RDTs, with J.Mitra-Dengue-ELISA being the most effective. In secondary infections, all IgM tests were more sensitive. These findings emphasise the superiority of ELISAs and testing for both NS1 and IgM markers for dengue diagnosis.

According to the findings of this study, the rapid antigen NS1 test has high sensitivity and positive predictive value but low specificity and negative predictive value. This finding is consistent with the previous studies by Blacksell and Dussart et al that found the rapid antigen NS1 test to be limited in diagnosing dengue.[14,15] It was also found that the quantitative ELISA test had higher sensitivity, specificity, and positive predictive value, than the negative predictive value. Similarly, Kumaraswamy et al and Osorio et al reported that the quantitative ELISA test had high accuracy in diagnosing dengue.[9,16] However, because the test has a low negative predictive value, it should be used with care to negate the presence of dengue.

CONCLUSION

The dengue NS1 qualitative ELISA test was found to have better specificity than the rapid antigen test with similar sensitivity indicating ELISA being better for diagnosis than the rapid tests. As quantitative ELISA tests are not readily available in low resource settings given their costs, both qualitative ELISA NS-1 and Rapid NS-1 can be acceptable for screening. The study has some limitations, including a small sample size and the use of a single-centre study design. To confirm the findings of this study, larger sample sizes and multicentre study designs are needed in future studies. Lastly, this study adds to our understanding of the diagnostic accuracy of the rapid antigen NS1 test and the quantitative ELISA test in the diagnosis of dengue. Therefore, aiding in developing effective strategies for dengue detection and management.

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