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Comparing the effectiveness of rapid Non-structural glycoprotein-1 (NS1) test antigen detection kit with IgM ELISA: A prospective study

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Abstract
Background: Early diagnosis of dengue virus (DV) infection is important to improve clinical outcomes. Non-structural glycoprotein-1 (NS1) has proven to be a useful biomarker for early diagnosis of dengue.
Aims and Objectives: To evaluate rapid NS1 antigen card test and compared with IgM ELISA for early dengue diagnosis in acute febrile illness.
Materials and Methods: Hundred patients with acute febrile illness were studied in the Department of Medicine Gandhi Medical College and associated Hamidia Hospital Bhopal. Patients were studied for NS1 antigen using rapid NS1 antigen detection kit and compared it with IgM ELISA test results. USG abdomen and Chest X-ray were also performed in each patients.
Results: Mean age of patients of acute febrile illness was 29.94±13.23 years with male male preponderance (61%). Majority had duration of fever for 4-5 days (35%). 64% patients were found positive for NS1 antigen. IgM dengue test was positive in 57% patients. Sensitivity and specificity of NS1 antigen was 53.1% and 36.1% respectively. Positive predicting value and negative predicting value of NS1 antigen test was 59.6% and 30.2% respectively.
Conclusion: Though insignificant NS1 antigen test had lower sensitivity and specificity compared to IgM ELISA test. Previous studies are in agreement with our findings. However, NS1 antigen test has several advantages over IgM ELISA test.

Keywords: acute febrile illness, Enzyme-linked immune sorbent assay, immunoglobulin, thrombocytopenia

Introduction

Infection of Dengue virus (DV) is considered as one of the most prevalent arthropod related disease though out the world. The effect of DV is more prevalent in the tropical and subtropical countries [1].

Patients with asymptomatic or undifferentiated fever with DV infection is called as dengue fever (DF) whereas patients with fever with plasma leakage are known as dengue hemorrhagic fever (DHF). In many of the patients DHF become more severe which may be lethal for the patients, especially children is known as dengue shock syndrome (DSS) [2].

In acute phase of DV infection, NS1 glycoprotein has been detected with intracellular organelles and also found to get transported through cellular secretion pathway over cell surface. Sera of patients infected with DV are also found to have NS1 glycoprotein [3].

Confirmation of presence of DV relies of the proper isolation of DV from serum, detection of single strand RNA using reverse transcriptase polymerase chain reaction and antibody detection which are specific to DV using enzyme linked immunosorbent assay (ELISA) [4].

All these test are several limitations. Antibody detection using ELISA is a costly affairs and is useful only during the early phase of infection as the antibodies are only detected only around the fifth day of DV infection [5]. Moreover, detection of immunoglobulin (IgM) is simply a indicative of a recent DV infection. IgM detection should not be considered as the confirmatory diagnosis of acute phase of DV infection.

Rapid antigen and antigen capture ELISA commercial kits had been developed and available for the detection of DV infection. This kit detects the NS1 antigen. NS1 antigen detection is considered as a new approach for the diagnosis of acute DV infection [6]. Data on the use of Rapid antigen NS1 detection in Indian population is limited. Hence in present study we tried to evaluate rapid NS1 antigen card test and compared with IgM ELISA for early dengue diagnosis in acute febrile illness.

Materials and Methods

Present Observational analytical cross sectional study was performed on 100 patients in the Department of Medicine Gandhi Medical College and associated Hamidia Hospital Bhopal for one year (April 2017 to March 2018).

Patients with high grade fever less than 5 days without any source of infection, with fever <5 days duration with thrombocytopenia and patients with fever <5 days with rashes of bleeding from any site were included. Patients of malaria, typhoid, URTI, LRTI and UTI, other sources of infection like meningitis ob abscesses, patients with history of fever>5 days and patients of leucocytosis were excluded from the present study. Approval from Institutional ethics committee was obtained before starting the study.

After a detailed sociodemographic details investigation including routine blood and urine examinations, rapid NS1

antigen detection kit: within 5 days of fever, IgM ELISA after 5th day of fever, USG abdomen, Chest X-ray, platelet count, malaria antigen and widal test were performed.
All the data analysis was performed using IBM Statistical Package for the Social Science (SPSS) ver. 20 software. Frequency distribution and cross tabulation was used to prepare the tables. Micro soft office 2010 was used to prepare graphs. Quantitative data was expressed as mean ± SD whereas categorical data was expressed as percentage. Descriptive analysis was used to obtain mean, SD, minimum and maximum values of variable. Chi square test was used to compare the categorical data. Level of significance was assessed at 5%.

Results

Mean age of study cohort 29.94±13.23 years. Majority of the patients were in the age group of 21-30 years (33%) followed by ≤20 years (32%) and 41-50 years (14%). Male preponderance was noted in present study (61%).
Most of the patients had duration of fever for 4-5 days (35%) followed by 2-3 days (33%) and 59% had rashes. Mean platelet and WBC was 146223±98994 and 6421.30±2548.95 respectively.
In present study, 64% patients were found positive for NS1 antigen and 36% were found negative for NS1 antigen. IgM dengue test was positive in 57% patients whereas it was negative in 43% patients.
USG findings revealed that 24% had ascites, 7% had renal parenchymal disease changes and 6%pleural effusion. Chest X Ray revealed that out of 100 patients, 6% had Pleural effusion.

Table 1: Comparing IgM with NS1 antigen test

Comparing IgM ELISA test with NS1 antigen card test		IGM dengue ELISA		Total	P value
		Negative	Positive		
NS1 antigen	Negative	13	23	36	0.297
	Positive	30	34	64	
Total		43	57	100	

IgM; Immunoglobulin M, ELISA; Enzyme-linked immune sorbent assay, NS1; Non-structural glycoprotein-1 P value of <0.05 is considered as significant

Table 2: Sensitivity and specificity of NS1 antigen card test

Sensitivity and specificity of NS1 antigen card test			IGM dengue		Total	P value
			Negative	Positive		
NS1 antigen	Negative	Count	13	23	36	0.297
		% within NS1 antigen	36.1	63.9	100.0	
	Positive	Count	30	34	64	
		% within NS1 antigen	46.9	53.1	100.0	
Total		Count	43	57	100	
		% within NS1 antigen	43.0%	57.0%	100.0%	

IgM; Immunoglobulin M, NS1; Non-structural glycoprotein-1 P value of <0.05 is considered as significant

Table 3: Positive and negative predicting value

Sensitivity and specificity of NS1 antigen card test			IGM dengue		Total	P value
			Negative	Positive		
NS1 antigen	Negative	Count	13	23	36	0.297
		% within IGM dengue	30.2	40.4	36.0	
	Positive	Count	30	34	64	
		% within IGM dengue	69.8	59.6	64.0	
Total		Count	43	57	100	
		% within IGM dengue	100	100	100	

IgM; Immunoglobulin M, NS1; Non-structural glycoprotein-1, P value of <0.05 is considered as significant

Discussion

Acute febrile illness comprises infection due to malaria, influenza, leptospirosis, scrub typhus, typhoid fever, and dengue, of which some infections necessitate specific treatment. As antibiotics will be required to treat bacterial diseases, knowing the viral etiology will help in avoiding unnecessary administration of antibiotics.
In view of high mortality and morbidity associated with dengue especially in tropical countries [7, 8]. It is imperative to diagnose the disease during the early phase in order to provide information for appropriate management and avoidance of complications. The NS1 antigen is found together with endothelium, free or soluble in the serum of patients, and can be detected on days 0-9 after the onset of symptoms [9, 10].
Dengue virus infection has emerged as a notable public health problem in India in recent decades and has become endemic with outbreaks occurring frequently and explosively almost annually [8].
The major diagnostic methods currently available are viral culture, viral RNA detection by Reverse Transcriptase Polymerase Chain Reaction (RTPCR) and serological tests such as Non Structural Protein (NS1), IgM Capture and IgG Capture ELISA (enzyme linked immunosorbent assay). However early dengue diagnosis still remains a major problem as all these assays have their own pitfalls. The first two assays have restricted scope as a routine diagnostic procedure. Viral isolation by immunofluorescence though a gold standard, cannot be used as a routine diagnostic procedure due to its low sensitivity, laborious procedure & time consumption.
In present study most common age group in present study

was 21-30 years (33%) followed by ≤20 years (32%) and 41-50 years (14%). Solanke *et al.* reported that the most common age group affected was 0-20 years, with 81.9% (127/155) samples belonging to this group [1]. Reports of Sharma *et al* showed that out of a total of 780 suspected cases of dengue, 101 cases (12.9%) tested positive with age ranging from 2 years old female child to 78 year old male. An increased incidence was observed in 2nd-4th decade (66.33%) followed by the 1st and 5th decade (25.74%) [11].

In present study maximum patients were male (61%) followed by female (39%). In a similar study including 155 samples of suspected dengue cases by Solanke *et al.* found female preponderance [1]. In agreement to present study findings reports of Sharma *et al.* also showed male preponderance [11].

In present study most of the patients had duration of fever for 4-5 days (35%) followed by 2-3 days (33%). Reports of Solanke *et al.* have also found that the maximum number of 56 samples was received during 1-3 days of fever, followed by 52 samples during 4-6 days of fever, and 47 samples during 7-9 days of fever [1].

It is important to diagnose dengue early with the help of specific and inexpensive diagnostic serological markers that would permit early intervention to treat patients and prevent or control epidemics as no vaccine is yet available for protection and the vector control measures are inadequate. Ideally, all new point of care diagnostics, including diagnostics for infectious diseases should meet the WHO ASSURED criteria. They should be: A- Affordable by those at risk of infection S- Sensitive with very few false negatives S- Specific with very few false positives U-User friendly tests that are simple to perform and require minimal training R-Rapid, to enable treatment at first visit [11].

On comparing patient distribution between NS1 Antigen card test and IgM ELISA test, we found that Out of 36 negative results in NS1 antigen test, 13 were also found to be negative in IgM ELISA test whereas 23 were found positive. Similarly out of 64 positive cases found in NS1 antigen test, 30 were found negative and 34 were found positive in IgM ELISA test.

Sensitivity and specificity tests are essential for accurate laboratory diagnosis of DENV infected patients. In present study out of 64 patients who were found positive in NS1 antigen test, 34 (53.1%) were also found positive in IgM ELISA test whereas out of 36 negative results in NS1 antigen test, 23 (63.9%) were found positive in IgM ELISA test. That means the sensitivity and specificity of NS1 antigen was 53.1% and 36.1% respectively. However the comparison was insignificant (p=0.297) Solanke *et al.* compared the performance of rapid NS1 antigen, IgM ELISA test, on serum 155 samples of suspected dengue cases and reported sensitivity of 55.5% and specificity of 92% [1]. Which is in agreement to present study findings where sensitivity obtained by Solanke *et al* is comparable to findings of present study. In a similar study by Dussart *et al*. showed that the NS1 antigen assay was more sensitive with samples collected up to day 3 after the onset of symptoms [12]. In agreement to that in present study maximum samples were corrected within 4 days of fever. A study by Najioullah *et al.* showed that the sensitivities of rapid NS1 antigen were 49.4% when compared to RT-PCR [13]. Similar results were also seen by Shrivastava *et al.* [14] Their evaluation showed an overall higher sensitivity rate for ELISA test as compared to NS1 antigen test for laboratory diagnosis of acute dengue infection [14]. However they also reported that ELISA assay takes longer, and requires a better equipped laboratory and technical expertise, it is considered as the confirmatory test

[15]. Sharma *et al*. studied the validity of card test against ELISA (gold standard) over a total 780 blood samples obtained for suspected dengue cases during monsoon and early post monsoon and reported that sensitivity and specificity of NS1 antigen test when compared with ELISA for detecting NS1 Ag was 100% while, ELISA was more sensitive, specific with higher diagnostic accuracy (100%) for detecting IgM and IgG antibodies as compared to NS1 antigen test [11].

In present study positive predicting value and negative predicting value of NS1 antigen test is 59.6% and 30.2% respectively. Solanke *et al.* in a similar report reported that positive and negative predictive values for rapid NS1 antigen were 78.9% and 79.4%, respectively [1].

Study by Stephen *et al.* [16] showed a sensitivity and specificity of 97.54% and 98.33%, respectively for SD RICT when compared with ELISA for detection of NS1Ag. Several other studies showed 100% specificity for detecting NS1 Ag by NS1 antigen test when compared with ELISA. However, the sensitivity of NS1 antigen test for NS1 Ag varied from 62.5% to 81.5% [12, 14]. Previous reports have shown that the sensitivity of the rapid kits was low to moderate. Usually, NS1 antigen has low sensitivity in case of secondary infection. NS1 levels may be quickly masked by circulating antibody and/or cleared from circulation. Hence, the most effective diagnostic application of NS1 detection is when it is combined with antibody detection. Together, they provide a broader window of detection [17]. Seok *et al.* showed a relatively lower sensitivity of 53.5% for IgM detection by NS1 antigen test. However, specificity and PPV were 100% each [18]. Sahu *et al.* [19] got a higher sensitivity and specificity of 93.90% and 99.53% in detecting IgM in early convalescent sera while Kaylan D, et.al have recorded a higher positivity with RICT than ELISA [20]. Though Dengue IgM Ab is a marker of recent infection it has cross-reactivity with other circulating Flaviviruses. Seok *et al.* proposed that SD Dengue Duo NS1/IgM combined can increase the sensitivity to 88.65% with specificity of 98.75%. So NS1 antigen test can be useful, sensitive, and specific for the diagnosis of acute dengue infection [18]. Moorthy *et al*. showed the sensitivity and specificity of NS1 antigen test for detection of IgM and IgG as 81.8% and 75% & 87.5% and 66.6%, respectively [21]. Several other studies showed differences in sensitivity and specificity of ELISA and rapid tests and their difference might be due to the different principles of these assays [22].

Cross sectional nature was the main limitation of present study. Hence findings of the present study cannot be applied to large population. Small sample size was another limitation; a large randomized clinical trial is needed to strengthen the present study findings.

Conclusion

We found a lower sensitivity and specificity of NS1 antigen test as compared to IgM ELISA test; however the comparison was insignificant and is in agreement to most of the previous studies performed. However NS1 antigen test is simple, point of care test for detection of NS1 Ag and dengue-specific IgM antibodies can be detected in a test card format with results available within 20 min. It does not require complicated technical expertise. It can be performed on blood. No pre-treatment of sera to remove competing IgG or rheumatoid factor is required. It can be performed on single or small number of samples and is cost effective.

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