Original Research Article

Comparative analysis of NS1 antigen card test and ELISA in clinically suspected dengue fever patients at a tertiary hospital

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Abstract

Background: Dengue infection often presents as an acute febrile illness and is endemic to the Indian sub-continent. Immunoassays for NS1 and IgM offer a convenient format for dengue diagnosis, and several enzyme-linked immunosorbent assay (ELISA) and rapid diagnostic tests (RDTs) are commercially available. In present study we aimed to compare results of NS1 antigen card test and ELISA in clinically suspected dengue fever patients. **Material and Methods:** Present study was single-center, comparative study conducted patients of all age groups, with clinical symptoms and signs of acute dengue like illness and whom serological diagnosis requested for dengue infection. Sensitivity, specificity, and predictive values were calculated for NS1, compared with ELISA. **Results:** In present study, during study period total 184 samples received in laboratory, satisfied study criteria and were considered for study. Males (59.78 %) outnumbered females (40.21 %), male to female ratio was 1.48:1. Most common age groups were 21-30 years (26.6 %) followed by 31-40 years (21.2 %). Out of total 184 samples, 79 samples tested positive by NS1 antigen card test (42.9 %) and 102 samples tested positive by NS1 ELISA (55.4 %). Sensitivity, specificity, PPV, NPV and accuracy when only NS1 was considered on rapid test kits when compared with ELISA were 76.04%, 93.18%, 92.41, 78.10 % and 84.24%. **Conclusion:** NS1 antigen card test in combination with ELISA assay offers the most sensitive and cost-effective diagnostic modality for dengue.

Keywords: NS1 antigen card test, NS1 ELISA, Dengue infection, Immunoassays

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Received Date: 19/10/2020 Revised Date: 12/11/2020 Accepted Date: 15/12/2020
DOI: https://doi.org/10.26611/10081914

Access this article online	
Quick Response Code:	Website: www.medpulse.in
	Accessed Date: 14 July 2021

INTRODUCTION

In India, the dengue incidence has increased in recent past and emerged as an important health problem in many states. Dengue, a viral infection often presents as an acute febrile illness and is endemic to the Indian sub-continent, caused by dengue virus, single-stranded, positive sense enveloped RNA virus belonging to the family Flaviviridae.[1] The dengue virus is found in serum or plasma, circulating blood cells and selected tissues especially those of the immune system, after the onset of illness (2 to 7 days), roughly corresponding to the period of the fever.[2] There is no specific treatment for dengue/ severe dengue, but early detection and access to proper medical care lowers fatality rates below one percent and helps in early patient management and immediate application of appropriate vector control methods which can help to prevent the spread and control of the infection. Presently, the basic methods used by most laboratories for the diagnosis of dengue virus infection include viral isolation, detection of viral genomic sequence by nucleic acid amplification technology assay (RT-PCR), Antigen detection, particularly non-structural protein 1 (NS1) and the detection of dengue virus-specific IgM antibodies by the IgM-capture enzyme linked immunosorbent assay

How to cite this article: Rajeshwari K G. Comparative analysis of NS1 antigen card test and ELISA in clinically suspected dengue fever patients at a tertiary hospital. *MedPulse International Journal of Microbiology*. July 2021;19(1): 19-22.
https://www.medpulse.in/Microbiology/

(MAC-ELISA) and/or the rapid dengue immunochromatographic test (ICT).[3] Immunoassays for NS1 and IgM offer a convenient format for dengue diagnosis, and several enzyme-linked immunosorbent assay (ELISA) and rapid diagnostic tests (RDTs) are commercially available. In present study we aimed to compare results of NS1 antigen card test and ELISA in clinically suspected dengue fever patients at a tertiary hospital.

MATERIAL AND METHODS

Present study was single-center, comparative study conducted in department of microbiology at Aster CMI hospital, Bengaluru, India, during period from January 2019 to December 2020 (2 year). Institutional ethical committee approval was taken prior to start of study.

Inclusion criteria

Patients of all age groups, with clinical symptoms and signs of acute dengue like illness and whom serological diagnosis requested for dengue infection

Exclusion criteria

Nonconclusive reports

Already diagnosed cases of dengue (referred or admitted with dengue positive report)

Primary details (age, sex, complaints, medical history) were noted in proforma. Blood samples were collected under aseptic precautions and serum was separated and stored for further analysis. The serum samples from the first group were subjected to NS1 antigen card test (SD BIOSENSOR Health care Limited, India) and NS1Ag capture ELISA (Panbio,Australia,)tests. The tests were performed strictly adhering to the kit manufactures instructions. Dengue NS1 antigen test utilizes the human serum / plasma followed by solid-phase immuno-chromatographic technology for the qualitative detection of dengue virus NS1 antigen. The membrane strip of the device is pre-coated with anti-dengue NS1 monoclonal antibody on the test region (T), and goat anti-mouse IgG is pre-coated on the control region (C). During testing, if the sample containing dengue NS1 Ag, the complex of the antibody-dengueNS1 Ag-gold conjugate moves laterally on the membrane by capillary action. In this case, the pink-purple line will appear on the membrane in test line (T). To serve as a procedural control, an additional line of Goat anti-mouse IgG has been immobilized on the card. If the test is performed correctly, this will result in the formation of pink purple line upon contact with the conjugate as a control line. Dengue NS1 ELISA kit uses one enzymatically amplified, two-step sandwich-type immunoassay to detect low levels of NS1 in serum. In this Dengue NS1 ELISA kit, controls and unknown serum samples are diluted in sample dilution buffer, containing secondary antibody, and incubated in microtitration wells. These wells on the Dengue NS1 ELISA kit have been coated with a highly effective NS1 antibody and then blocked. NS1 antigens present in the samples on this Dengue NS1 ELISA kit are then sandwiched between the capture and secondary antibodies. The presence of NS1 antigen on this Dengue NS1 ELISA kit is confirmed by the colorimetric response obtained using an enzyme-conjugate-HRP and liquid TMB substrate. The patients who tested positive for NS1 Ag or IgM antibody by ELISA were taken as confirmed cases and to be suffering from acute dengue infection. Statistical analysis was done using descriptive statistics. Sensitivity, specificity, and predictive values were calculated for NS1, compared with ELISA.

RESULTS

In present study, during study period total 184 samples received in laboratory, satisfied study criteria and were considered for study. Males (59.78 %) outnumbered females (40.21 %), male to female ratio was 1.48:1. Most common age groups were 21-30 years (26.6 %) followed by 31-40 years (21.2 %).

Table 1: Age and Gender wise distribution

Age Group (Years)	Males	%	Females	%	Total	(%)
0-10	9	4.9	5	2.7	14	7.6
11-20	11	6.0	8	4.3	19	10.3
21-30	29	15.8	20	10.9	49	26.6
31-40	22	12.0	17	9.2	39	21.2
41-50	19	10.3	12	6.5	31	16.8
51-60	13	7.1	8	4.3	21	11.4
>61	7	3.8	4	2.2	11	6.0
Total	**110**	**59.78**	**74**	**40.21**	**184**	**100**

Out of total 184 samples, 79 samples tested positive by NS1 antigen card test (42.9 %) and 102 samples tested positive by NS1 ELISA (55.4 %).

Table 2: Comparison of result by different diagnostic assays

Diagnostic test (n=184)	No. of dengue positive samples	Percentage
NS1 antigen card test	79	42.9
NS1 ELISA	102	55.4

Sensitivity, specificity, PPV, NPV and accuracy when only NS1 was considered on rapid test kits when compared with ELISA were 76.04%, 93.18%, 92.41, 78.10 % and 84.24%.

Table 3: Sensitivity, specificity, and predictive values

NS1 ELISA	NS1 antigen card test		Total
	POSITIVE	NEGATIVE	
POSITIVE	73	6	79
NEGATIVE	82	23	105
Sensitivity			76.04
Specificity			93.18
Positive Predictive Value			92.41
Negative Predictive Value			78.10
Accuracy			84.24

DISCUSSION

There is no prevention in the form of any vaccine for dengue, thus early diagnosis and treatment is recommended for preventing complications and disease control in the endemic regions. In addition to difficulties with prevention of dengue, definitive diagnosis of the infection has also proven to be difficult because its symptoms are non-specific, especially in the early, acute stage of the infection. The precise diagnosis of dengue infection can be achieved through viral isolation, viral RNA detection through RT-PCR, but this methods is time consuming, costly and not within the reach of even most of the tertiary care hospitals, so its diagnosis is based on the detection of dengue specific antibodies and/or NS1 antigen or ELISA. NS1 (DENV Non-structural protein 1) found in both membrane and soluble forms which is highly conserved. The NS1 antigen is highly specific and detectable in serum from days 1 to 9 after fever onset;[4] its sensitivity depends on the type of test used and the time since onset of symptoms (it declines in parallel with viraemia), and is higher in primary than secondary dengue.[5,6] While IgM antibodies level increases rapidly and appears to peak about 2 weeks after the onset of symptoms, then decreases to undetectable levels over 2–3 months.[7] In present study majority of patients were predominantly males (59.78 %) than females (40.21 %). Similar observations were noted by Raju BJ *et al.*[8] (males 61% over females 39%) and by Dash PK. *et al.*[9] Anand AM *et al.*,[10] studied 112 clinically suspected dengue cases, 94 were laboratory-confirmed dengue cases (positive by one or more of the following tests - IgM ELISA, NS1 antigen ELISA and RTPCR). The positive detection rate of NS1 antigen ELISA, RT-PCR and IgM ELISA were 80.9%, 68.1% and 47.9% respectively. NS1 antigen ELISA was evaluated using RT-PCR as the reference standard and showed a sensitivity of 96.8%, specificity of 53.3%, positive predictive value of 81.6% and negative predictive value of 88.9%. The combination of NS1 and IgM had the highest sensitivity of 97.8%. In study by Mahesh Kumar *et al.*,[11] Overall prevalence of dengue infection was 45.7%. Among 116 suspected dengue cases, 25% were positive by NS1 ICT, 29.3% were positive by NS1 ELISA and 37.9% were positive by IgM MAC-ELISA. The sensitivity of NS1 ICT was 52.27% and specificity was 91.66% whereas sensitivity for ELISA was 56.81% and specificity was 87.5%.[11] Similar findings were noted in present study. Otta S *et al.*,[12] noted that on rapid test, 78 cases were NS1 antigen positive of which 60 cases were positive only for NS1 antigen. When NS1 rapid and ELISA tests when compared, 16 kit negative tests were positive on ELISA while 34 kit positive tests were ELISA negative. Sensitivity, specificity, PPV and NPV when only NS1 was considered on rapid test kits when compared with ELISA were 78.9%, 87.8%, 63.8% and 93.8%. Tabasum B *et al.*, studied 228 serum samples from patients with suspected dengue infection were subjected to rapid Immunochromatographic card test (ICT) and IgM-Capture ELISA (MAC-ELISA). The sensitivity and specificity of ICT was 66.24% and 90.14% while it was 93.69% and 54.70% for MAC-ELISA. Dengue NS1 antigen detection through immunochromatographic rapid card test along with MAC-ELISA proves to be more sensitive in the early diagnosis of dengue virus infection. The primary infection is indicated by the presence of NS1 antigen alone or IgM alone and presence of NS1+IgM whereas the secondary infection is indicated by the presence of IgG alone and NS1+IgG as NS1 appears early in both primary and secondary infection. NS1 protein is highly conservative for all the serotypes of dengue and they circulate in high levels in the blood during the first few days of the illness owing to their long half-life in blood.[14] Even with this advantage, prevalent reports regarding detection of NS1 in the

presence of antibodies are conflicting in nature.[15] A multi-country evaluation study reported that the best performing NS1 assay had only a moderate sensitivity (median 64%, range 34-76%), with 100% specificity. The poor sensitivity of the evaluated assay has been related to study sites in different geographical regions suggesting the need for further assessment.[16] Also prolonged antibody responses to many infections preclude the use of most serological rapid diagnostic tests (RDTs) for monitoring response to treatment and/or for diagnosing relapse. NS1 antigen detection had the highest sensitivity in the early stages while IgM detection was more sensitive in the latter half of the illness.[10] NS1 ELISA showed a slightly better sensitivity but specificity of NS1 ELISA was higher. However, for early diagnosis and management of acute dengue, both NS1 antigen and IgM antibody detection tests need to be used in conjunction.

CONCLUSION

NS1 antigen card test in combination with ELISA assay offers the most sensitive and cost-effective diagnostic modality for dengue. Dengue NS1 antigen test is designed for primary screening test of dengue virus NS1 antigen, can provide fast and easy way to get a result but cannot completely exclude the possibility of false positive or negative result caused by various factors.

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Source of Support: None Declared
Conflict of Interest: None Declared