## Full Length Research Article

# DETECTION OF *NS1* ANTIGEN FROM SUSPECTED DENGUE VIRAL INFECTION CASES BY CAPTURE ELISA AND IMMUNOCHROMATOGRAPHY METHODS: A COMPARAITIVE STUDY

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## ABSTRACT

**Background:** Dengue virus infection is one of the serious mosquito borne viral infection mainly affecting the population from tropical and subtropical countries of the world with high morbidity and mortality. In India it is a major public health problem with all four serotypes of Dengue virus (DENV-1, DENV-2, DENV-3, DENV-4). An estimated 50 million dengue infections occur annually. Among the hospitalized 2.5% of the children die with disease. There is no immunoprophylaxis or specific antiviral therapy available so far. Therefore early, timely and reliable diagnosis is important in patient management, implementation of control measures and for epidemiological purposes.

**Aims and objectives:** Aim of this study is to detect NS1 antigen, anti-dengue Ig M antibody by ELISA and Immunochromatography (ICT) methods from a single serum sample of clinically suspected cases of dengue virus infection and also to determine the reliability of ELISA and ICT methods to detect the NS1 antigen.

**Materials and methods:** Total 100 serum samples were collected from clinically suspected and were subjected to NS1, IgM ELISA and NS1, IgM ICT.

**Results:** Among the 100 serum samples collected, 42 were positive by NS1 ELISA, 70were positive by IgM ELISA and 74 were positive by combination of NS1 and IgM ELISA. The sensitivity and specificity of NS1 ELISA was 42% and 100%, whereas NS1 ICT was 46% and 100% respectively. The sensitivity, specificity of IgM ELISA were 94.5% and100% and IgM ICT were 51.4% and 100%.

**Conclusion:** Combination of antigen and antibody assays on a single sample from a clinically suspected cases is preferable for making a reliable diagnosis. A positive result by ICT suggests dengue infection but a negative result does not rule out the infection which is a major obstacle considering this test as the test of choice for the diagnosis of clinically suspected case of dengue.

## INTRODUCTION

Dengue fever infection has become a major international public health concern in the recent past. Over the past three decades, there has been global increase and occurred in many epidemic forms. It is one of the serious mosquito borne viral infection mainly affecting the tropical and subtropical countries of the world (World Health Organization, 2011). An estimated 50 million dengue infections occur annually among them nearly 500000 cases are being hospitalized with DHF. A very large proportion of them are children and about 2.5% of those affected die (Park, 2013). There is no immunoprophylaxis or specific anti viral therapy available for dengue virus infection.

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Therefore early and timely diagnosis is important in patient management and implementation of control measures (Shrivastava *et al*., 2011). There are four DENV serotypes, DENV-1, DENV-2, DENV-3, DENV-4 which are distinct and closely related to each other antigenitically and may results in antigenic cross reactivity (Roehrig *et al*., 2005). But subsequent infections of two serotypes leads to more severe form of disease (Bhatia *et al*., 2013). Dengue viruses are mosquito (*Aedes aegypti)* borne flavi viruses and the only arbo virus fully adapted to a human- mosquito- human cycle and no longer depend on the forest cycle for maintenance (Roehrig *et al*., 2005). These mosquitoes lives in urban areas and breed mostly in manmade containers (Bhatia *et al*., 2013). Transovarial transmission of the virus exists in the vectors and maintains the virus in nature (Joshi *et al*., 2002). The NS1 (a type of non structural protein) is highly conserved glycoprotein and has got major role in the replication of viral RNA and its pathogenesis.

The role of NS1 in virus replication is clearly not known but believed to facilitate viral infection and DENV pathogenesis.NS1 is in addition secreted from infected cells and has been shown to be immunologically important. It is also of diagnostic importance (Back and Lundkvist, 2013). It is possible that NS1 contributes directly to the vascular permeability syndrome. Dengue NS1 interacts with the complement system and the complement split products have been considered as central to vascular permeability syndrome. Dengue virus infection shows wide variety of clinical presentations like acute febrile illness of 2-7 days duration, headache, retro orbital pain, myalgia, arthralgia, rash, hemorrhagic manifestations, leucopenia, rising hematocrit (World Health Organisation, 2009).

It may be asymptomatic or may presents as undifferentiated febrile illness or dengue hemorrhagic fever including dengue shock syndrome. The clinical manifestations depend on the virus strain and host factors such as age, immune status (World Health Organization, 2011). DENV-1 infection results in more severe clinical appearance thaninfection with DENV-4 (Nishiura and Halstead, 2007). NS1 antigen detection in the sera of patients with dengue virus infection during acute phase of illness from the first day of illnessappears to be  highly specific (Alcon *et al*., 2002 and Dussart *et al*., 2006). Detection of NS1 Ag by ELISA has been found to be a useful tool (Datta and Wattal, 2010 and Wang and Sekaran, 2010). Since the dengue fever is an urban and semi-urban areas, there is a constraint for health care system to utilize the services of diagnostic laboratories with great technological backup. So,test should be simple to perform and easy to interpret in the form of immunochromatography (ICT) based rapid tests (Kulkarni *et al*., 2011)

## MATERIALS AND METHODS

This prospective study was conducted over a period of one year from June 2013 to June 2014. During this period 100 clinical samples were  received from Narayana General and Super speciality hospital to the department of microbiology, Narayana Medical College. Patients with clinical history of fever,  nausea, vomiting, rash and body pains were admitted with suspicion of dengue virus infection for evaluation. About 5ml of blood was collected in a vacutainer tubes by venipuncture under strict aseptic precautions. The blood was allowed to clot. After retraction of clot, specimen was centrifuged at 3000 rpm for 5minutes.Serum was then transferred to a labeled container to subject it for NS1 ELISA and NS1 Immunochromatography later.

### NS1 antigen detection by ELISA and Dengue IgM capture ELISA

SD Dengue NS1 antigen ELISA kit was used for qualitative detection of NSI antigen and SD Dengue IgM capture ELISA was used for detection of IgM antibodies against dengue virus. The test was performed according to the kit manufacturers instructions in automated ELISA processor which was programmed for the assay procedure and calculation and interpretation of the results obtained. The work list is prepared in the computer system. All the reagents were bring to room temperature before testing.

*Procedure*

100µl of controls and samples were added to their respective wells. Then the wells were incubated at 37ºC for 60 minutes. The wells were washed 5 times with 350µl of washing solution, giving 10 seconds soak time for each wash and all the contents were aspirated from the wells.100µl of enzyme conjugate was added to each well and mixed well on vibrating mixer. Then the wells were incubated at 37ºC for 60 min then washed for 5 times. 100µl of working TMB solution was pipetted out in each well and allowed to react for 10 minutes at room temperature.100µl of stop solution was added to each well. The absorbance of the wells was read with at 450nm and 620nm wavelength within 30minutes after adding stop solution.

*Interpretation*

### NS1 antigen detection by ELISA

The mean absorbance of the negative controls was 0.091. Cut off value =0.009+0.3 = 0.391 (mean absorbance of NC+0.3).Samples with < cutoff value were considered as negative and more than were positive.

### Dengue IgM capture ELISA

The mean absorbance of the negative controls was 0.103,Cut off value = 0.103+0.3 = 0.403 (mean absorbance of NC+0.3). Samples with <cutoff value were negative,and more were positive.

### Immunochromatographic method for detection of NS1 antigen

The SD BIOLOINE Dengue rapid kit was used. This is one step assay designed to detect dengue virus NS1 antigen in human serum, plasma or whole blood.

*Procedure*

With a disposable dropper add 3 drops of serum into sample well. As the test begins to work, purple colour moves across the result window. Test results were interpreted at 15- 20 min.

*Interpretation*

The presence of one color line (C band) within the result window indicates negative result. Whereas presence of two color lines (T band and C band) within the result window indicates positive result. If the color line was not visible within the result window, then the test was considered as invalid.

## RESULTS

Among the 86 serum samples processed, 37(43%) were positive by NS1 ELISA, 60(69.8%) were positive by IgM ELISA and 64(74.4%) were positive by combination of NS1 and IgM ELISA. The sensitivity and specificity of NS1 ELISA are 57.8% and 100%, for NS1 ICT was 64% and 100% respectively. The sensitivity and specificity of Ig M ELISA

was 93.7% and 100% same parameters for IgM ICT are 50% and 100% respectively.

### 1. Diagnosis of the clinically suspected cases of dengue virus infection

| Total | Dengue positive | Dengue negative |
|---|---|---|
| 100 | 74 | 26 |

The table shows that out of the 100 clinically suspected cases,74 cases were Dengue positive and 26 cases were Dengue negative after correlation with NS1 + IgM ELISA.

### 2. Day of fever when sample was collected

| Day of fever when sample was collected | Number of patients | NS1 positive | IgM positive |
|---|---|---|---|
| Day 2 | 2 | 2 | 0 |
| Day 3 | 11 | 7 | 8 |
| Day 4 | 31 | 16 | 21 |
| Day 5 | 32 | 11 | 20 |
| Day 6 | 15 | 4 | 12 |
| Day 7 | 09 | 2 | 7 |
| Total | 100 | 42 | 70 |

This table shows that NS1 antigen will be detectable from day 2 till day 7 of fever, whereas IgM antibody was detected from day 3 only to day 7.

### 3. Correlation between ELISA and ICT in NS1 detection

| Assay | | NS1 ELISA | | Total |
|---|---|---|---|---|
| | | Positive | Negative | |
| NS1 ICT | Positive | 42 | 4 | 46 |
| | Negative | 0 | 54 | 54 |
| Total | | 42 | 58 | 100 |

This table shows Correlation between ELISA and ICT in NS1 detection in which positivity by NS1 ELISA was 42 and NS1 ICT was 46 samples out of 100 samples

### 4. Sensitivity and specificity of ELISA v/s ICT in detecting NS1 antigen

| Parameter | ELISA | ICT |
|---|---|---|
| Sensitivity | 42% | 46% |
| Specificity | 100% | 100% |

Table shows sensitivity of NS1 ELISA to be lower than NS1 ICT. However this difference is not statistically significant and there is good correlation between ELISA and ICT methods in detecting NS1 antigen.

### 5. NS1 ELISA v/s combination of NS1 ELISA and IgM ELISA(n=86)

| Assay | | NS1 ELISA + IgM ELISA | | Total |
|---|---|---|---|---|
| | | Positive | Negative | |
| NS1 ELISA | Positive | 42 | 0 | 42 |
| | Negative | 32 | 26 | 58 |
| Total | | 74 | 26 | 100 |

The table shows the difference between results of NS1 ELISA and combination of NS1 ELISA and IgM ELISA in suspected cases is significant.

### 6. IgM ELISA v/s combination of NS1 ELISA and IgMELISA (n=86)

| Assay | | NS1 ELISA + IgM ELISA | | Total |
|---|---|---|---|---|
| | | Positive | Negative | |
| IgM ELISA | Positive | 70 | 0 | 70 |
| | Negative | 4 | 26 | 30 |
| Total | | 74 | 26 | 100 |

The table shows the difference between results of IgM ELISA and combination of NS1 ELISA and IgM ELISA in suspected cases. Combination is slightly better than single test.

### 7. Sensitivity and specificity of ELISA v/s ICT in detecting IgM antibody

| Parameter | ELISA | ICT |
|---|---|---|
| Sensitivity | (70/74)94.5% | (36/70)51.4% |
| Specificity | 100% | 100% |

## 8. Positivity of NS1 ELISA, IgM ELISA and combination of NS1 ELISA and IgM ELISA

| Assay | Positivity |
|---|---|
| NS1 ELISA | 42 |
| IgM ELISA | 70 |
| NS1 + IgM ELISA | 74 |

## DISCUSSION

Dengue fever is an acute febrile arboviralillness affecting the tropical and subtropical regions of the world. The incidence of the this disease has increased over the last 50 years with high morbidity and mortality. It is therefore imperative to have a rapid and sensitive laboratory test\ for the early detection. Out of 100 cases studied, 74 were dengue positive and 26 were dengue negative. In the present study, NS1 antigen was found to be present from day 2 till day 7 of illness and IgM was found from day 3 of the illness. According to a study by *Alcon et al* in 2002, NS1 antigen could be detected from day 1 till day 9 of illness (Alcon *et al*., 2002). *Wang et al* in 2010 study shows NS1 antigen may be detected upto day 14 of illness and IgM will be found from day 3 of illness (Wang and Sekaran, 2010). In the present study out of 100 samples, NS1 ELISA was positive in 42 cases, IgM ELISA was positive in 70 cases. Out of 42 cases where NS1 antigen was positive, concomitantly IgM was also detected in 32 cases.

Hence there is reasonably significant increase in the detection of dengue cases when NS1 ELISA was added to IgM ELISA. In our study the sensitivity, specificity of NS1 ELISA were 42% and 100%. But, *Sekaran et al*, 2010 study by SD Dengue NS1 Ag ELISA showed the assay had a sensitivity of 76.76% and specificity of 98.31%. However the sensitivity dropped due to increased concentration of antibodies. In the present study, sensitivity, specificity of NS1 ICT was 46%,100% respectively. A study from Duong *et al* in 2010, showed overall sensitivity and specificity of NS1 Ag kit to be 57.5% and 100% respectively (Duong *et al*., 2011). The authors have concluded that the NS1 antigen results should be interpreted with caution when used alone. NS1 Ag assay has to be combined with IgM antibody capture ELISA, which significantly can increase the sensitivity for dengue diagnosis. Hence the authors recommend the use of combination of NS1 antigen assay and IgM capture ELISA for better diagnosis dengue illness. Studies by Kumaraswamy *et al*., 2007, shown that dengue NS1 Ag ELISA to be more sensitive for diagnosis in the acute phase of primary infection than the secondary infection.

### Conclusion

Since laboratory assay in isolation is not adequate enough to diagnose all cases of dengue virus infection. Combination of antigen and antibody testing on a single serum sample from a clinically suspected dengue cases is more appropriate for making a reliable diagnosis.NS1 positive and IgM positive result by ICT is highly suggestive of dengue infection but a negative result doesn't rule out the infection. According to the findings of the present study and in correlation with the studies done by so many other researchers, it can be inferred that for a patient presenting with dengue like symptoms in a endemic or epidemic region, a NS1 positive result by ICT highly suggestive of dengue infection.

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