

Original Research Article

Evaluation of NS1, IgM ELISA and RT-PCR in diagnosis of dengue fever

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ABSTRACT

Background: Dengue fever is a mosquito borne disease caused by *flavivirus*. Its cases are increasing in India with increasing mortality rate year by year hence, prompt and accurate diagnosis is necessary to prevent morbidity and mortality.

Methods: In this study we enrolled 125 clinically suspected cases of dengue. All the collected samples were processed for RT-PCR, NS1 and IgM ELISA. We evaluated NS1 antigen ELISA alone, and combination of NS1 and IgM ELISA against RT-PCR.

Results: Among 125 clinically suspected case 67 were positive by RT-PCR and 58 were negative. Sensitivity and Specificity of NS1 ELISA and NS1 with IgM ELISA (in combination) against RT-PCR were 83.58%, 94.82% and 95.55%, 79.31% respectively. (p<0.001).

Conclusions: The NS1 ELISA alone was sufficient to detect acute phase of dengue fever, although, combination of NS1 and IgM proved to be most appropriate method for detection of acute as well as late phase of dengue fever.

Keywords: Dengue, IgM ELISA, NS1 ELISA, RT-PCR

INTRODUCTION

Dengue is a mosquito borne viral infection, transmitted by mosquito species, *Aedes aegypti* and *Aedes albopictus*. Dengue fever (DF), sometimes complicates and result into severe dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS). Dengue virus has four different serotypes. All these serotypes have the capacity of causing full spectrum of disease. The diagnosis of dengue is mainly based on ELISA detecting either NS1 antigen or IgM antibody capture (MAC IgM) in countries where dengue is prevalent.^{1,2} The patients with dengue fever have high levels of nonstructural protein-1 (NS1) protein in their serum after onset till 1-7 days. Therefore, NS1

could be useful diagnostic indicator for acute dengue fever.³

However, anti-dengue virus IgM is produced during both primary and secondary infection. After 3-4 days of infection, IgM rises rapidly and is usually identified after 5-6 days. Its titre reaches the peak at about 14 days of infection, and then declines to undetectable levels over 2-3 months. In secondary infections, the IgM concentrations are maximal around 2 weeks after acute infection and then become undetectable by 4 weeks.⁴ On other hand the molecular methods like reverse transcriptase polymerase chain reaction (RT PCR) for diagnosis is promising for early detection with its serotypes. This test has been approved by WHO in

dengue bulletin 2009.⁵ The current study was undertaken with the aim to diagnose cases of dengue fever and to determine the diagnostic utility of NS1 Antigen ELISA and IgM ELISA at acute phase of illness to help prompt management.

METHODS

Study design and site

It is a cross sectional study conducted at Viral Research and Diagnostic Laboratory (VRDL), Department of Microbiology, Uttar Pradesh University of Medical Sciences, Saifai, Etawah, UP, India. The consent was taken from each patients during enrolment in this study. The inclusion and exclusion criteria were followed as mentioned below:

Inclusion criteria

- Age: All age groups.
- Clinically suspected cases of dengue fever visiting to O.P.D. and I.P.D. of different clinical department of UPUMS, Saifai, Etawah, UP.

Exclusion criteria

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

Study period, sample collection and processing

A total of 125 cases were enrolled with clinical signs and symptoms (headache, fever, joint pain, etc.) suggestive of dengue fever during outbreak (July 2016 to Jan 2017). 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 and molecular diagnosis.

Patients signs and symptoms

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

NS1 antigen ELISA

Non-structural (NS1) antigen ELISA was performed using Qualisa™ Dengue NS1 kit as per manufacturer protocol, (Qualpro Diagnostics, Verna, Goa).

Dengue IgM antibody capture (MAC) ELISA

Dengue antibody detection was done by MAC IgM ELISA kit as per manufacturer instructions, (Calbiotech, El Cajon, California).

RNA extraction and real time reverse transcriptase PCR

The RNA was extracted by the standard procedure as per manufacturer protocol (AccuPrep® viral RNA extraction kit, bioneer corporation, Korea). 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 (Applied Biosystem™, Carlsbad, California).

RESULTS

Of the total 125 clinically suspected dengue cases, 79 were confirmed cases (either positive by RT-PCR, NS1 ELISA or IgM ELISA).

Among 79 positive cases 52 were male and 27 were female, with M: F ratio 1.9:1. In this study most common symptom was fever present in all cases 79 (100%), followed by arthralgia 51 (64.46%) cases, other clinical signs and symptoms are shown in Figure 1.

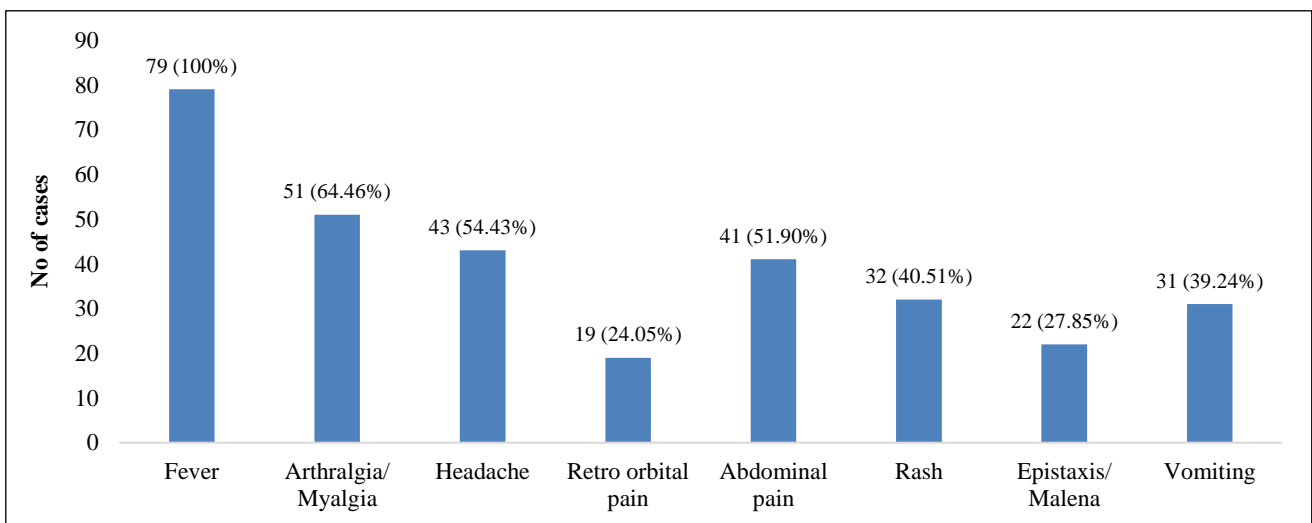


Figure 1: Clinical sign and symptom in dengue positive cases (n=79).

Among all the cases, 67 cases were positive by RT-PCR, 59 cases were positive by NS1 antigen ELISA and 47 cases positive by IgM antibody capture ELISA. A total of 76 cases were detected by serological methods NS1 and IgM ELISA.

Among 67 RT-PCR positive cases, 56 cases were found NS1 positive and 11 cases were negative. Thus the 83.58% sensitivity, 94.91% positive predictive value were analysed. Among 58 RT-PCR Negative cases, only 3 cases were found positive by NS1 and 55 cases were

found to NS1 negative with the 94.82% specificity and 83.3% negative predictive value ($p < 0.001$), (Table 1).

Similarly, the sensitivity and specificity were analysed for the combined result of NS1 and IgM against RT-PCR. Among all the positive 67 cases of RT-PCR, 64 cases were detected by serologically using a test in combination (NS1 and IgM ELISA). Both ELISA detected 12 cases additionally which were negative by RT-PCR. We found 95.55% sensitivity and 79.31% specificity of serological test ($p < 0.001$) (Table 1).

Table 1: Comparison of NS1 ELISA, NS1 ELISA and IgM (in combination) against RT-PCR.

TESTX	RT-PCR		Sensitivity	Specificity	PPV	NPV	χ^2
	POS.	NEG.					
NS1 ELISA	POS.	56	83.58%	94.82%	94.91%	83.33%	$\chi^2-73.58$, DF-1, P<0.001*
	NEG.	11					
NS1 ELISA+ IGM ELISA	POS.	64	95.55%	79.31%	84.21%	93.87%	$\chi^2-69.94$, DF-1, P<0.001*
	NEG.	3					

PPV= Positive predictive value, NPV= Negative predictive value. df -degree of freedom, * yates correction

DISCUSSION

Dengue fever is an emerging disease associated with high morbidity and mortality. The diagnosis is still great challenge in developing country due to lack of resources, infrastructure and skilled manpower. A recent study published shows that the prevalence of dengue fever occurred in increasing order of each year.⁶ This disease is a major health problem in India and needs to be diagnosed and treated in early phase of the disease to avoid any associated morbidity and mortality.

In this study, 52 cases were males and 27 females, among all the 79 total positive cases. Thus, male preponderance was seen which might be due to more outdoor activities of males as compared to females. Like our study, a study from north India also demonstrated that the males were more commonly affected than females.⁷

The clinical profile of dengue reveal that fever was the most common presenting symptom 79 (100%) in our study. Our results also coincides with the study by Damodar et al.⁸ In the present study, the other common symptoms next to fever, were, arthralgia/myalgia, 51 cases (64.56%), headache 43 cases (54.43%) and abdominal pain 41 cases (51.90%). Similar results were also reported by Goel et al, and Anuradha et al.^{9,10}

Retro orbital pain is considered as an cardinal sign in clinical diagnosis of dengue. An Indian study by Laul et al., reported 41% patients of dengue have retro orbital pain. However, in our study only 19 (24.05%) of cases have this symptom. Rashes were found in 32 (40.51%)

cases in our study, but other studies have reported it as a less common symptom occurring in about 21% cases only.^{11,12} Its seems that the trend of clinical presentation is changing year by year in dengue patients with increasing severity.

The effective and accurate diagnosis of dengue is of primary importance for clinical care and management. The laboratory diagnostic methods for confirming the diagnosis may involve detection of the virus, viral nucleic acid, antigens or antibodies, or a combination of these techniques. After the onset of illness, the virus can be detected in serum, plasma, circulating blood cells and other tissues for 1-15 days.¹³ The serological methods, like NS1 and IgM ELISA are routinely being used worldwide. Here in this study we also used NS1 and IgM ELISA both as the main diagnostic tool. The sensitivity and specificity for NS1 antigen ELISA against RT-PCR was found to be as 83.58% and 94.82% respectively in our study with p value as ($p < 0.001$). A recent Indian study also supports our higher positivity rate of NS1 as they found that positive detection rate of NS1 antigen ELISA was 80.9%.¹⁴

The NS1 and IgM ELISA methods are showing a higher sensitivity and specificity either alone or in combination of both test. In our study NS1 and IgM ELISA sensitivity and specificity is 95.55% and 79.31%. Many reports show their higher sensitivity and specificity range from 53-96%.¹⁴⁻¹⁶

The maximum positivity of RT PCR was observed in NS1 positive samples, which was showing a strong

correlation between them. It is due to high viremia in initial days. Therefore the 83.58% sensitivity and 94.82% specificity was observed by NS1 ELISA.

CONCLUSION

In conclusion, the NS1 ELISA alone was sufficient to detect acute phase of dengue fever, however, combination of NS1 and IgM ELISA proved most appropriate method for detection of acute as well as late phase of dengue fever.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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