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Evaluation of NS1 Antigen Detection for Early Diagnosis of Dengue Virus Infection in a Tertiary Care Hospital in Karnataka, India

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A B S T R A C T

K e y w o r d s Dengue diagnosis, NS1 antigen assay, MAC ELISA, acute phase, convalescent phase. **Article Info** *Accepted:* 26 November 2016 *Available Online:* 10 December 2016	To evaluate the efficacy of NS1 antigen as a early marker for diagnosis of Dengue virus infection. Performance of NS1 antigen assay in comparison to MAC ELISA was evaluated when performed together in a single sample in Group 1. 391 acute/early convalescent sera were screened by both the assays. Group 2 evaluated the specificity of NS1 assay in comparison to MAC ELISA on 30 samples included as controls. In Group 1, 162(41.43%) were positive by NS1 antigen assay and 71(18.15%) were positive by MAC ELISA. NS1 antigen positivity decreased from 96.91% in acute phase sera to 3.08% in early convalescent sera ($P < 0.0001$). Conversely IgM positivity increased from 7.04% in acute phase sera to 92.95% in early convalescent sera ($P < 0.0001$). All the samples in Group 2 were negative showing 100% specificity of both the assays. NS1 Ag is an effective tool for the diagnosis of DENV infection, especially with in first 7 days of illness. Combined use of NS1 antigen assay with MAC ELISA test could significantly improve diagnostic sensitivity of dengue infection.

Introduction

Dengue has become a major global public health problem in the developing countries. The estimated risk of acquiring Dengue virus infection (DV) is approximately 2.5 billion people living mainly in urban areas (Halstead *et al.,* 2007). DV causes various clinical symptoms ranging from asymptomatic or undifferentiated fever, known as dengue fever (DF), dengue hemorrhagic fever (DHF), dengue shock syndrome (DSS), leading to death, especially among children (Gubler *et al.,*1999).

Viral isolation by culture or viral RNA detection by PCR helps in the diagnosis of recent dengue infection. But this viral isolation is very time consuming and requires specialized laboratory equipment (Sathish *et al.,* 2003). Newer PCR types like nested reverse transcription-PCR (RT-PCR) and realtime RT-PCR have significantly re-duced processing times but are expensive and technically exacting (Bessof *et al.,* 2008). As a result, dengue culture and PCR have limited utility in routine clinical use.

Rapid newer test for presumptive diagnosis of dengue is detection of NS1 (Non structural protein) Ag. This antigen is a highly conserved glycoprotein that is essential for the viability of dengue virus (Dussart *et al.,* 2006). The first immunoglobulin isotype to appear is IgM antibody, suggesting recent infection. One of the most recent advances for routine dengue diagnosis is IgM antibody capture ELISA (MAC ELISA) (Vaughn *et al.,* 2000). To confirm dengue during both early and late infection, combined usage of NS1 antigen and IgM antibody ELISA are promising (Andries *et al.,* 2012).

Here, in this study, we have performed both types of immunoassays; NS1 ELISA and IgM ELISA, in the samples received in our laboratory and the results of the combined tests have been compared individually with each test separately.

Materials and Methods

The comparative study was conducted in Government hospital as a part of Integrated Disease Surveillance Programme (IDSP) in Raichur, Karnataka from January 2015 to December 2015. Demographic details of the patients were collected. Depending on the reporting time of the patients, sera comprised of both acute and early convalescent phases. Samples were divided into two Groups ie Group 1 and Group 2.

Group 1 was the test group which included all patients (both adults and children) with fever fitting into WHO revised classification of dengue (WHO, 2009). The samples were screened for the presence of dengue-specific IgM antibody capture enzyme linked immunsorbent assay(MAC-ELISA),using a kit prepared by the National Institute of virology, Pune, India, strictly following the manufacurer's protocol (Cecilia *et al.,*2011).

NS1 Ag was detected in the sera by PanBio NS1Ag ELISA kit. Data was statistically analyzed by calculating the 'p' value.

Group 2 was the control group which included 30 patients. 20 were obtained from patients with fever due to known etiology other than dengue [enteric fever (12), bacterial meningitis (3), UTI (5)] and 10 were from healthy blood donors. All these 30 were screened by both the assays.

Results and Discussion

Out of the 391 samples in Group 1, 162 (41.43%) samples were positive for NS1 Ag and 71 (18.15%) were positive for IgM antibody including those that were positive by both (Table 1).

157 were from acute phase sera and 5 were from early convalescent phase out of 162 NS1Ag positive samples. The NS1 Ag detection rate decreased from 96.91% in acute phase sera to 3.08% in early convalescent sera ($P < 0.0001$). All the 122 samples that were positive for NS1 Ag alone in this study group I belonged to acute phase sera (Table 2).

66 were from early convalescent phase and only 10 were from acute phase sera out of 71 MAC ELISA positive samples. All the 31 samples that were only IgM positive belonged to early convalescent phase (Table 2). IgM antibody detection rate increased from 7.04% to 92.95% ($P < 0.0001$).

Total number of male patients was 210 (53.70) and female patients were 181 (46.29%). Out of 391, highest were from 1-10 yr age group, followed by 11-20 yrs and lowest were > 61 yr age group (Fig 1).

Fig 2 depicts details of both IgM negative & NS1 negative patients.

Serum samples which were positive by both NS1 antigen ELISA & IgM ELISA were also highest in the age group of 1-10 yrs. (Fig :3).

Fig 4 shows serum samples that were positive only by IgM.

The IgM capture ELISA is most commonly used in India due to its low cost and ease of handling. But here it is important to understand that, NS1 antigen detection assay has an advantage over IgM detection that it can diagnose a case of dengue while the latter cannot, because IgM and IgG antibodies remain detectable for months after the clinical illness and hence test results obtained from single sera are only suggestive of infection (Andries *et al.,* 2012). To confirm a case of acute dengue infection by serology, IgM seroconversion or a fourfold increase of IgG antibody titer in paired sera must be demonstrated (Tricou *et al.,*2010).

In the present study, 31.20% sera were positive only for NS1antigen, by ELISA.

Several studies have reported a lesser values of 23.3% (Datta *et al.,*2010), 30% (Kulkarni *et al.,* 2011) as well as higher value of even 60 % (Santosh Tathe *et al.,* 2013) positivity exclusively for NS1 antigen for diagnosis of dengue. (Table 1)

In this study, 7.92% cases were positive for IgM only and 10.23% cases were positive for both NS1 antigen and IgM antibodies. This clearly concludes that we would have missed a few positive cases if only NS1 antigen or only IgM antibodies were detected. Similarly, various studies also report a significant increase in detection of dengue when both the assays were performed together in a single sample (Schilling *et al.,* 2004 and Ampaiwan *et al.,* 2008) (Table 1). Our findings are also similar to a study done by Fauziah Md *et al.* which found that on 208 dengue suspected fever cases, NS1 antigen was positive in 67 patients (32.2%) and a total of 107 patients (51.4%) were positive for IgM and IgG antibodies while a combination of these tests would raise the detection of dengue fever in 129 cases out of 208 patients (62%).

Table.1 Detection Rate of MAC-ELISA and NS1 Assay in Dengue Positive Samples of Group I (n=391)

	NS1 antigen ELISA		Total
	Negative	**Positive**	
MAC ELISA	198 (50.63%)	122(31.20%)	320(81.84%)
Negative	31(7.92%)	40(10.23%)	71(18.15%)
Positive			
Total	229(58.56%)	162(41.43%)	391(100%)

Table.2 Positivity of NS1 antigen and IgM in Early (1-7 days) and Convalescent (8-14 days) phases

Day post onset of illness	NS1	IgM
1 to 7 days	**157/162 (96.91%)**	**5/71(7.04%)**
8 to 14 days	**5/162(3.08%)**	**66/71(92.95%)**

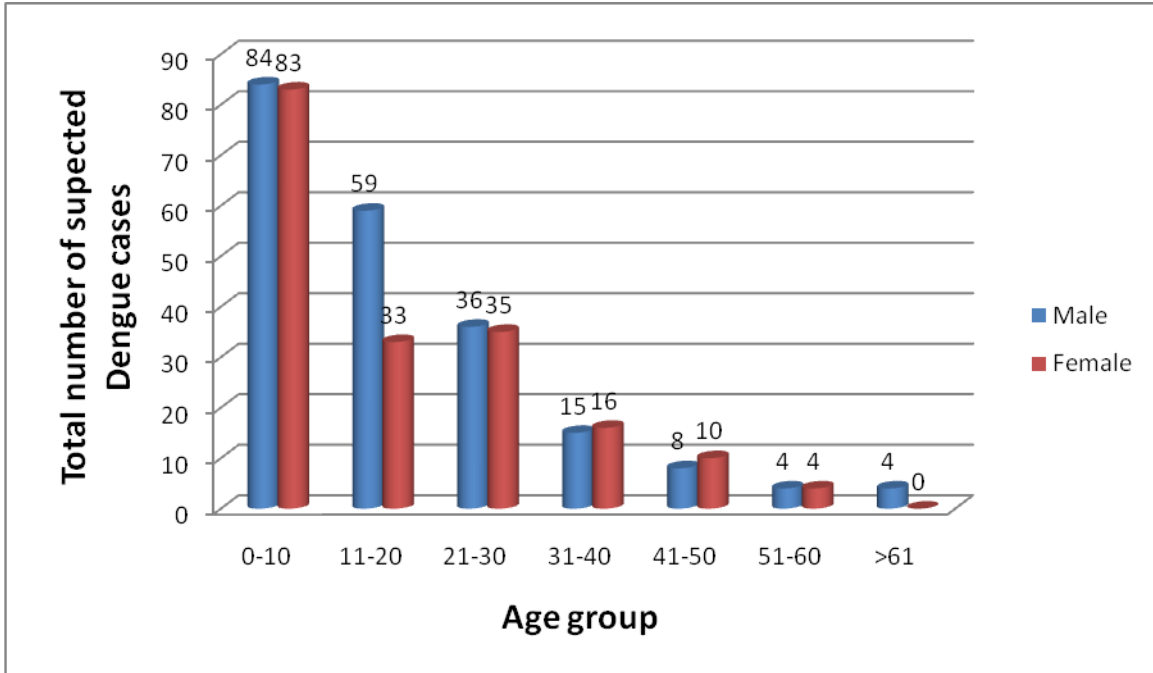**Fig.1** Showing total no. of suspected Dengue cases at different age group & gender.

Fig.2 Showing total no. of Dengue negative cases at different age group & gender.
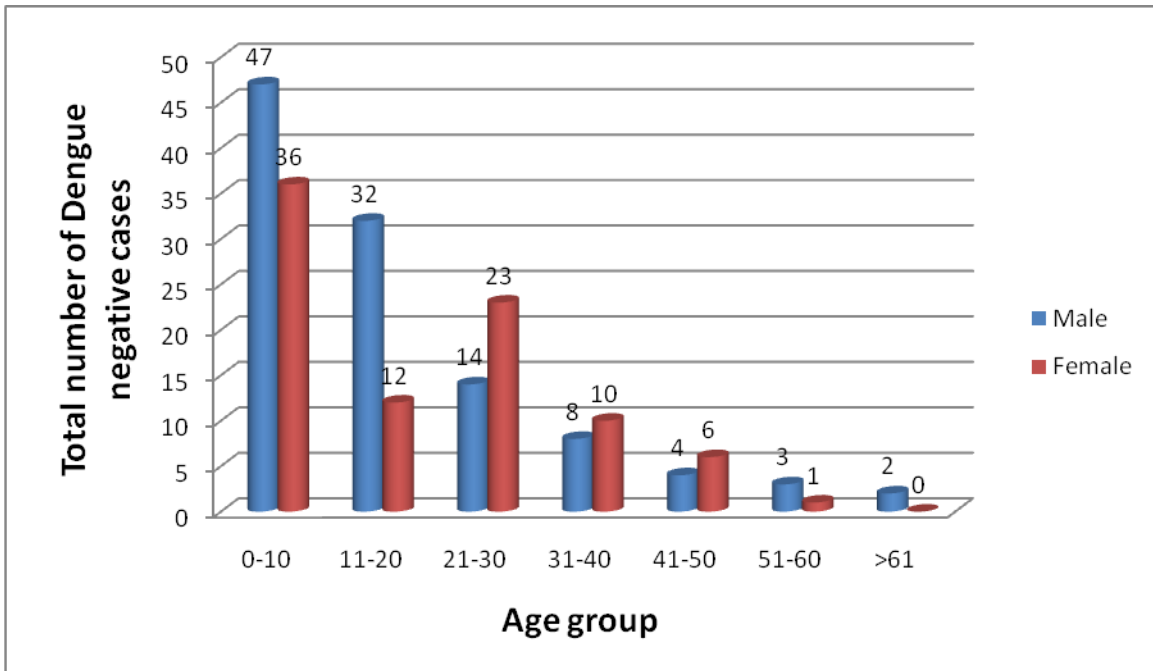
Fig.3 Showing total no. of IgM positive & NS1 positive cases at different age group & gender
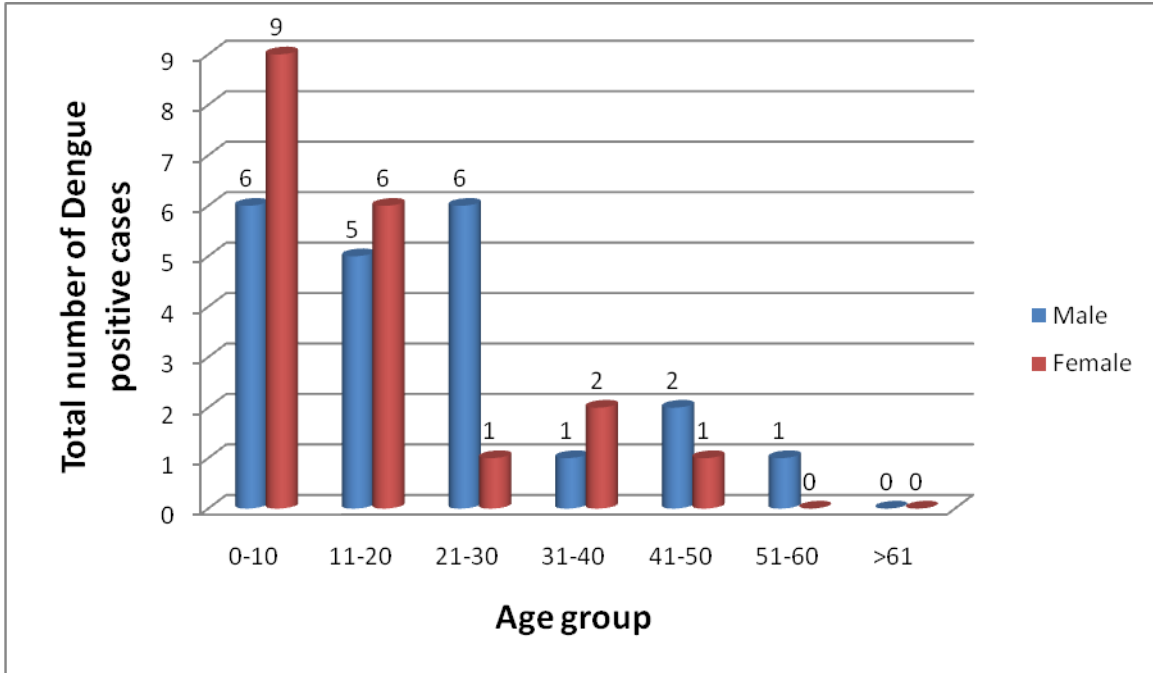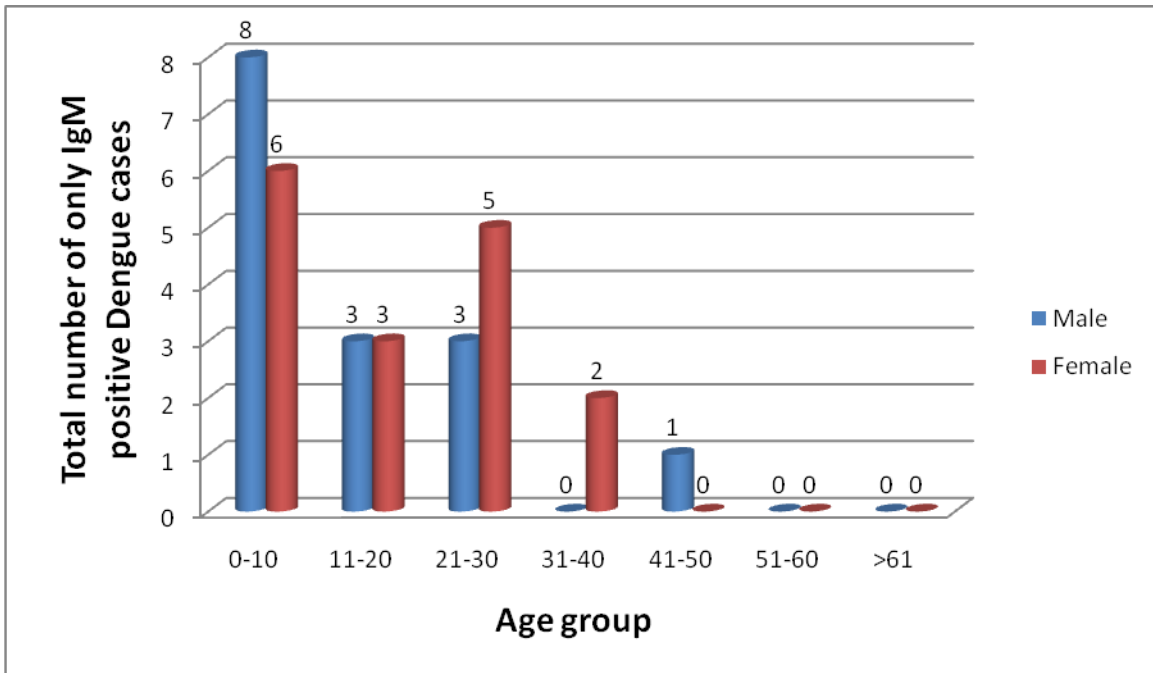

Fig.4 Showing total no. of IgM positive & NS1 negative cases at different age group & gender

Therefore the dengue NS1 antigen test can be used to complement the current antibody detection tests and the combination of these serological tests would increase the diagnosis efficiency of early diagnosis of dengue illness (Kassim *et al.,*2011) (Table 2).

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 shows NS1 antigen may be detected upto day 14 of illness and IgM will be found from day 3 of illness (Wang *et al.,* 2010) (Table 2). Studies by Kumaraswamy *et al.,* 2007, showed that dengue NS1 Ag ELISA to be more sensitive for diagnosis in the acute phase of primary infection than the secondary infection (Kumaraswamy *et al.,* 2007) (Table 2).

In our study, the highest number of cases belonged to the younger age group and males clearly outnumbered females which was in concordance with Gupta *et al*., and Chakravarti *et al.,* study which also showed maximum cases in age group of 21-30 years and male prepordance (Gupta *et al.,* 2006 and Chakravarti *et al.,* 2005). The young adults getting more affected reflects the presence of non-immune adult population falling prey to the circulating serotype of dengue virus and involvement in outdoor activities making them more exposed to mosquito bite. Contrasting results were reported by Madhulika *et al*., 2013 with highest prevalence in females (Madhulika *et al.,* 2013) (Table.3, 4, 5, 6, 7).

In conclusion, our finding suggest that the NS1 antigen assay is very useful and specific tool for the diagnosis of acute dengue infection. However, the sensitivity of the NS1 assay is depended on the level of viremia and host humoral immune response Therefore, combined use of NS1 antigen with dengue IgM test could significantly improve diagnostic sensitivity of dengue infection. Hence by doing both NS1 antigen detection test and IgM antibody detection test, we can diagnose dengue fever early so

that the morbidity and mortality can be reduced. We also conclude that NS1 Ag is an effective tool for the diagnosis of DENV infection, especially with in first 7 days of illness.

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Conflict of Interest: None to declare.

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