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Original Research Article

Prevalence and Diagnosis of Dengue in a Tertiary Care Hospital

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ABSTRACT

Background: Dengue is an acute febrile illness affecting the tropical and subtropical regions of the world. The incidence of this disease has increased over the last 50 years with 2.5 billion people living in areas where dengue is endemic.

Materials & Methods: The study was conducted in the Department of Microbiology, GCRG Institute of Medical Science. All clinically suspected cases of dengue during the period from July 2016 to October 2016 were included in the study. Serum samples were collected and tested for NS1, IgM, IgG by KIT and confirmation of the test was done by ELISA.

Results: Total 970 samples were tested, 789 were positive for one or more dengue parameters. Out of the 789, 573 were positive for NS1, 147 were positive for IgM, 69 were positive for IgG.

Conclusion: Detection of NS1 Antigen helps in early diagnosis of dengue. Combination of NS1 Antigen and IgM/ IgG can be used as predictor to reduce the morbidity and mortality of dengue disease.

Keywords: Dengue, Prevalence, Diagnosis, NS1.

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INTRODUCTION

Dengue fever is an arbo-viral disease affecting the tropical and subtropical regions of the world. The event of this disease has increased over the last 50 years with 2.5 billion people living in endemic areas.[1] Serious complications include Dengue Haemorrhagic Fever (DHF) and dengue shock syndrome (DSS) are found more in children now-a-days increasing disease burden.[2] DHF with death rate in most countries is 5%, primarily among young children and adults.[3] Rapid and sensitive laboratory methods required for early detection of the disease to reduce the morbidity and mortality.[4]

Mostly antibody (IgG/M) detection are commonly used for prognostic of dengue infection, but time required for appearance of IgM antibody is approximately 4-6 days.[5] Dengue non-structural 1 antigen (NS1) is highly conserved glycoprotein produced in both membrane associated and secretory forms is used as a new biomarker for early diagnosis of dengue infection.[6] An ideal dengue diagnostics would be rapid, simple, with high sensitivity and specificity, preferably able to differentiate between primary and secondary infections, as well as to serotype the viruses. Taking into account cultural differences and diverse customs of seeking medical attention when one is sick, the optimal time frame for diagnosis would be from the onset of dengue symptoms to 10 days post-infection.[6] Nevertheless, not all are able to be diagnosed within this time frame, as i) some people consult the physician only when in dire situations, ii) many people in third world countries rely heavily on traditional healing, iii) 2% of world population do not seroconvert and iv) there is a high number of dengue asymptomatic cases.[7]

Virus detection has always been a standard method in many pyrexial illnesses, with highly specific results. In dengue, the most applied cell lines for infection are the C6/36 mosquito cell line, Vero cell line and baby hamster kidney (BHK)- 21 cell line. More often than not, sera from dengue patients during the febrile phase are used for virus isolation, nevertheless, the virus has been traditionally isolated from plasma, whole blood and autopsy tissues in dengue cases.[8]

A confirmation assay that includes immunofluorescence or RT-PCR is performed once cytopathic effect is noted. Nevertheless, this method is tedious, and takes a long time (7 -- 12 days) before the virus is detected. Further, virus isolation desire cell culture facilities that may not be available in many endemic countries. The method also relies heavily on the virus survival in samples, directly affecting the time frame and proper storage of testing materials, as temperature may affect virus viability.

Despite virus detection being unsuitable for early diagnosis of dengue, it remains very useful and relevant as a diagnostic tool. It allows monitoring of dengue epidemiology and evolution as well as antigenic drift.[9]

The serological diagnostics are the most applied methods to diagnose dengue patients in many dengue endemic countries.

However, these serological tests are best utilized when dengue virus titers decrease and antibodies start forming. Dengue IgM

has been shown to appear from the seventh day of illness onward with 70% positivity and by the tenth day, the patient is expected to achieve 100% positivity of dengue antibodies, as the increase in IgM is shown to be directly proportional to the number of days after infection.[10]

MATERIALS AND METHODS

The study was conducted in the Department of Microbiology in GCRG Institute of Medical Science, Lucknow, UP, India. All clinically suspected cases of dengue during the period from July 2016 to October 2016 were included in the study. Serum samples were collected and tested for NS1, IgM, IgG by KIT and confirmation of the test was done by ELISA.

Inclusion Criteria

Clinically suspected patients experiencing febrile illness consistent with dengue fever with two more of the following manifestations:

- Headache
- Muscle pain
- Haemorrhagic manifestation
- Retro-orbital pain
- Rash

A total number of 900 acute phase blood samples were collected from children and adults age ranging from 0 to 80 years. WHO criteria were followed for inclusion or exclusion of cases of dengue infection and their categorization as DF/DHF.[9]

Sample Collection

5 ml of blood samples were collected from all the suspects as a part of the routine laboratory work and the serum were separated and tested for the Dengue NS1 and IgM by Viral markers and compared with ELISA methods.

Seroassays

NS1 Ag assay

NS1Ag MICROLISA (J. Mitra & Co, New Delhi) test kit was used to perform the test. NS1Ag MICROLISA is a solid phase enzyme linked immunosorbent assay (ELISA) based on the "Direct Sandwich" principle.

Anti-Dengue NS1 antibodies are coated on Microwells with high reactivity for Dengue NS1 antigen. The samples are added in the wells and then enzyme conjugate (monoclonal anti-dengue NS1 antibodies linked to Horseradish peroxidase (HRPO) added. A sandwich complex is formed in the well wherein dengue NS1 (from serum sample) is ''trapped'' or ''sandwiched'' between the antibody and antibody HRPO conjugate. Wash buffer is added that will washed off Unbound conjugate.

The amount of bound peroxidase is proportional to the concentration of dengue NS1 antigen present in the sample. Upon addition of the substrate buffer and chromogen, a blue colour develops. The intensity of developed blue colour is proportional to the concentration of dengue NS1 antigen in sample. Stop solution is added to limit the enzyme-substrate reaction, and a yellow colour develops which is finally read at 450 nm spectrophotometrically. Sample results were expressed in terms of ratio within 2 hours. As per the manufacturer's guideline, interpretation was done as (i) non-reactive for dengue virus NS1 Ag if ratio < 9, (ii) equivocal for dengue virus NS1 Ag if between 9 to 11, and (iii) reactive for dengue virus NS1 Ag if > 11 or more was obtained.

Detection of IgM & IgG

All the samples were tested for the presence of dengue specific IgM & IgG antibodies by using ELISA.

Table 1: Comparison of efficacy of various dengue specific parameters in the diagnosis of dengue infection.

Parameters	Total positive	Percentage
NS-1	573	(72.6%)
IgM	147	(18.6%)
IgG	69	(8.7%)
TOTAL	789	(81.3%)

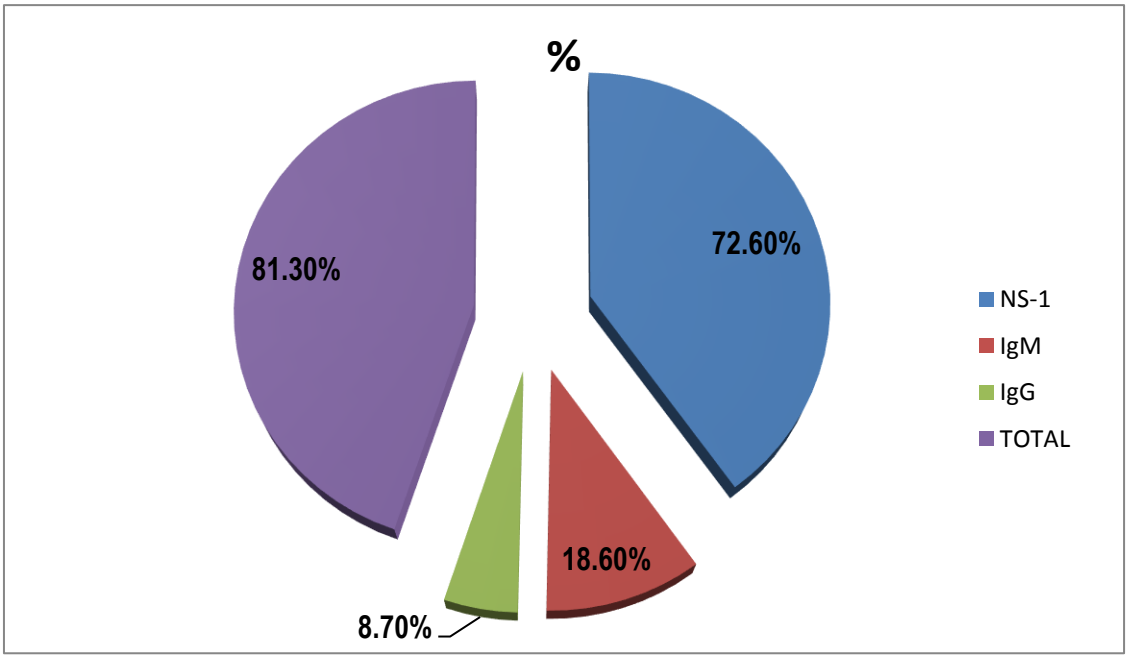

Fig 1: Comparison of efficacy of various dengue specific parameters in the diagnosis of dengue infection.

Table 2: Details of Age groups in positive test results.

Age groups (Years)	NS1 Positive	IgM Positive	IgG Positive
0-10	56	8	11
11-20	155	45	18
21-30	153	54	9
31-40	80	23	4
41-50	44	9	9
51-60	44	5	8
>60	41	3	10

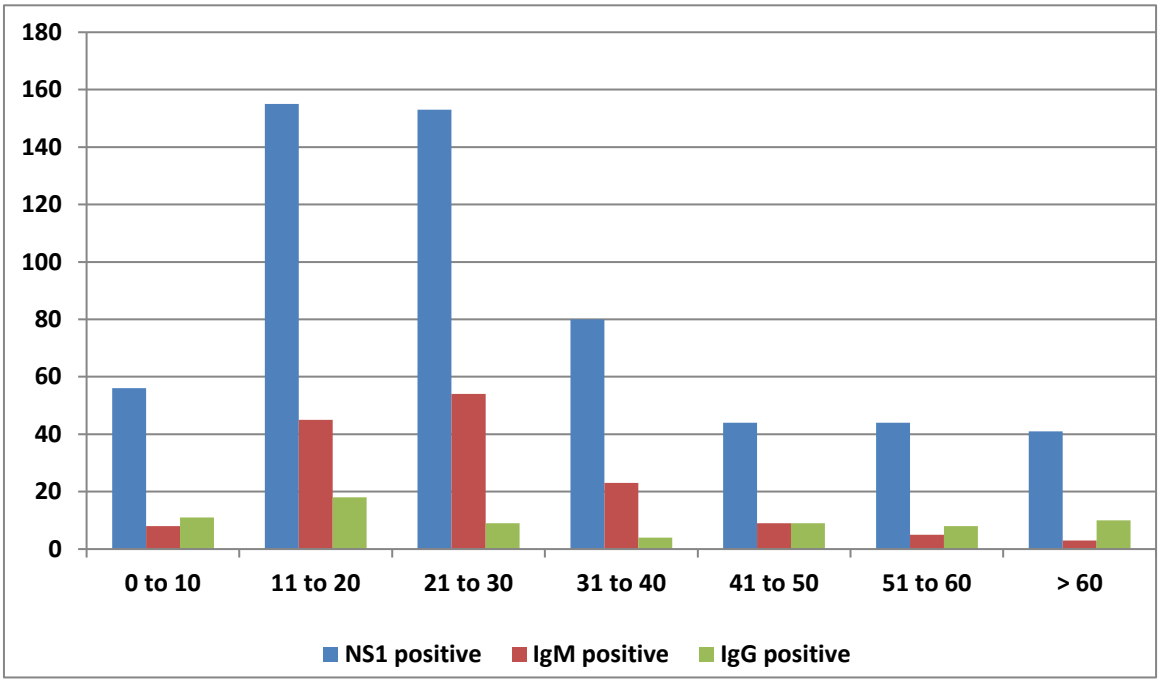

Fig 2: Details of Age groups in positive test results.

RESULTS

Out of total 970 samples tested, 620 were tested for NS1 and 350 samples were tested for IgM. Out of total 620 samples tested for NS1, 440 were males and 180 were females and out of total 350 samples tested for IgM and IgG, 210 were males and 140 were females. So out of total 970 samples tested male character 650(67%) was there than female 320 (32.9%).

Out Of total 970 samples tested a total of 789 (81.3%) samples were positive for either one or more of the markers (NS1 and IgM and IgG) tested. Of the 789 positive serum samples, 573 (72.6%) were positive for NS1 only, 147 (18.6%) were positive for IgM only and 69(8.7%) were positive for IgG.

DISCUSSION

Dengue is the most important arthropod-borne viral disease of public health significance, the global prevalence of Dengue has grown dramatically in recent decades with estimated 2.5 billion people at a risk of acquiring this viral infection and more than 50 million new Dengue infections being projected annually.[11] Dengue and its severe manifestations: DHF and dengue shock syndrome (DSS), are recognized as important emerging public health problems in tropics and subtropics, thats why it is more important to diagnosis dengue in early period with the help of clinical parameter like platelet counts and serological markers to reduce the morbidity and mortality in dengue.

Table 3: Comparison of studies regarding association of NS1 and IgM with Dengue specific parameters.

	Present study	Study of R.D. Kulkarni et al., [12]	Study of Krunal D. Mehta et al., [13]
No. of total cases tested	970	2104	1628
No. of positive cases	789	320	563
No. of cases positive for NS-1 only	573 (72.6%)	95(30%)	363(64.5%)
No. of cases positive for IgM only	147 (18.6%)	161(50%)	200(35.5%)

Investigation of dengue specific IgM/IgG is the mainly used for the detection of dengue infection since from long time. Mostly antibodies begin to appear on fifth day of fever in primary infection. But in some cases IgM/IgG antibodies cannot be detected before the third day of fever in secondary dengue infection. Therefore, there is some lag period both in primary and secondary dengue when test will give negative results in antibodies specific tests. For early diagnosis of DI the NS1

antigens, is available now a days from day 1 of fever both in primary and secondary infections. When NS1 is positive, there is no need of repeat testing as it is a highly specific marker of Dengue infection.[12,14,15]

This supports the fact that a large number of cases would be missed if NS1 is not included in the test panel.[12] The results of various dengue specific parameters are shown in [Table1]. Of the 789 cases which were tested for NS1 antigen only, 573 were positive only for NS1 antigen. It stated that 66.05% cases were diagnosed early preventing severe complication if we had not included NS1 antigen in the testing panel.

Earlier IgM ELISA and IgG ELISA were widely used diagnostic method for dengue fever in routine laboratory practice. Among the two antibodies, IgG is less reliable marker in the diagnosis of dengue infection as both clinical and subclinical infection can produce IgG which may persist for several years affecting the interpretation of testing results. IgG levels could be higher in endemic areas due to continues biting from mosquitoes so, dengue specific IgM is very good marker for acute cases and it may also give idea about secondary dengue infection.[14] In the endemic areas, dengue infection is mainly confirmed by rising titre in paired serology. However, repeat testing for the same infection when first sample was negative or for confirmation of dengue infection is almost not possible in routine clinical practise. When NS1 antigen gives positive results in first four days of illness, there is no need of repeat testing as NS1 is highly specific marker for the diagnosis of dengue infection.[14]

In study of R.D. Kulkarni et al.[12] total 2014 samples were tested, out of which 320 were positive for one or more dengue parameters. Of the total 320 positive cases, 95 (30%) were positive for NS-1 only, 161 (50%) were positive for IgM only. This study also tried to find the association of dengue parameter with thrombocytopenia. As the NS1 antigen is earliest marker detectable in blood from day one after onset of fever, its assay is an effective tool for early diagnosis so as to avoid complications of dengue infection. Out of these dengue specific parameters, platelet count is the only laboratory parameter performed in remote areas because of cost effectiveness and easy to perform without requiring costly setup, which can support the diagnosis of dengue infection. Such predictions will help to decrease complication due to late treatment and initiate the preventive and control measures well in time for the containment of spread of the disease. Therefore, studies like this will contribute significantly to the clinical management and can reduce morbidity and mortality in dengue infection.

CONCLUSION

The study draws attention toward the male, young adult age group. Inclusion of NS1 in the diagnosis of dengue increases the early diagnosis so as to avoid complications significantly. Detection of NS1 Antigen helps in early diagnosis of dengue. Combination of NS1 Antigen and IgM/ IgG can be used as predictor to reduce the morbidity and mortality of dengue disease. Whenever IgM is detected as compared to NS1 and can be used as an indicator to reduce the complication of dengue disease. The results of this study indicate that to prevent complication in dengue infection and early diagnosis more programmed study with correlation of other important clinical parameter and serological marker is needed in larger study group.

REFERENCES

1. Smith AW, Chen LH, Massad E, Wilson ME. Threat of dengue to blood safety in dengue-endemic countries. Emerg Infect Dis. 2009;15:8-11.

2. Whitehead SS, Blaney JE, Durbin AP, Murphy BR. Prospects for dengue virus vaccine. Nat Rev Microbiol. 2007; 5:518-28.

3. World Health Organisation. Key Issues in dengue vector control towards the opererationalisation of a global strategy: report of consultation. Geneva: WHO; 1995.(CTD/FIL(DEN)/IC.96.1).

4. Datta S, Wattal C. Dengue NS1 antigen detection: A useful tool in early diagnosis of dengue virus infection. Indian J Med Microbiol. 2010; 28:107-10.

5. World Health Organization. 1997. Dengue haemorrhagic fever: [6]Diagnosis, treatment, prevention and control. 2nd edition. Geneva, Switzerland. Chapter 4, Laboratory Diagnosis.

6. Subedi D and Taylor-Robinson AW. Laboratory diagnosis of dengue infection: current techniques and future strategies. Open Journal of Clinical Diagnostics. 2014; 4: 63-70.

7. Dussart P, Baril L, Petit L, et al. Clinical and virological study of dengue cases and the members of their households: the multinational DENFRAME Project. PLoSNegl Trop Dis 2012;6(1):e1482.

8. Rosen L, Drouet MT, Deubel V. Detection of dengue virus RNA by reverse transcription-polymerase chain reaction in the liver and lymphoid organs but not in the brain in fatal human infection. Am J Trop Med Hyg 1999;61(5):720-4.

9. Jarman RG, Nisalak A, Anderson KB, et al. Factors influencing dengue virus isolation by C6/36 cell culture and mosquito inoculation of nested PCR-positive clinical samples. Am J Trop Med Hyg 2011;84(2):218-23.

10. Shu PY, Huang JH. Current advances in dengue diagnosis. Clin Diagn Lab Immunol 2004;11(4):642-50.

11. Chakravarti A, Matlani M, Kashyap B, Kumar A. Awareness of changing trends in epidemiology of dengue fever is essential for epidemiological surveillance. Indian J Med Microbiol. 2012;30:222-26.

12. Kulkarni RD, Patil SS, Ajantha GS, Upadhya AK, Kalabhavi AS, Subhada RM et al. Association of platelet count and serological markers of dengue infection – importance of NS1 antigen. Indian Journal of Medical Microbiology. 2011; 29(4): 359-62.

13. Krunal D. Mehta, Bindiya Ghediya, Shruti sheth, Suraj Khandhediya, Hitesh Shingala, Mala Sinha et al. Study of Correlation Between Platelet Count and Serological Markers of Dengue Infection with Importance of NS1 Antigen in Western Region of India; National Journal of Laboratory Medicine. 2016 Apr, Vol 5(2): 55-59.

14. Peeling RW, Artsob H, Pelegrino JL, Buchy P, Cardosa MJ, Devi S, et al. Evaluation of diagnostic tests: Dengue. Nat Rev Microbiol. 2010;8: S30-37.

15. Golia S, Hittinahalli V, Sujatha K, Reddy M, Kamath AS. Serodiagnosis of Dengue using NS1 antigen, dengue IgM, dengue IgG antibody with correlation of platelet count. International journal of A J institute of medical sciences. 2012 ;1(2):112.

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