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Original Article

A STUDY OF DIAGNOSTIC APPLICABILITY OF NS1 AND IGM ELISA IN DENGUE FEVER IN A TERTIARY CARE HOSPITAL, VISAKHAPATNAM

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ABSTRACT

Objective: The present study aims at diagnostic applicability of NS1 antigen and IgM antibody detection in Dengue fever by ELISA method.

Methods: A prospective study for a period of one year from June 2023 to June 2024 was carried out on 13531 blood samples received from inpatients aged between 03 to 45 y admitted with a history of acute phase of fever>99.5°F, myalgia, arthralgia for more than 3 days duration. All the samples received were processed in Virology Research and Diagnostic Laboratory, Department of Microbiology, Andhra Medical College, King George Hospital, Visakhapatnam. Blood samples received were centrifuged @3000 rpm for 10 min, serum seperated and those single sera samples were tested for both NS1 antigen and IgM antibody by ELISA methodology. Cutoff values were calculated according to kit insert instructions. Biochemical laboratory parameters and prognostic markers like platelet count was also taken into consideration in the study.

Results: Positivity in this study was 16.3% (2212 of 13531). OD reading more than cutoff value was interpreted as positive or reactive. Detection range varied from 100% by NS1 Ag and 88% by IgM Ab ELISA, thus inferring sensitivity and specificity of respective procedures.

Conclusion: Epidemic episodes of dengue fever are frequently reported during monsoon. Hence, combination of NS1 and IgM on single serum samples can improve the diagnostic accuracy during acute phase of dengue fever and help in initiation of therapy and epidemic control to reduce morbidity and mortality.

Keywords: Dengue fever, ELISA, NS1 antigen, IgM antibody, Single sera sample, Epidemic

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INTRODUCTION

Dengue fever is an arthropod-borne, single-stranded RNA virus belonging to Flaviviridae, transmitted from mosquitoes to human. Two known mosquito species are female Aedes aegypti and Aedes albopictus. According to World Health Organization, globally around 50 million people are infected with the dengue virus annually [1]. It presents clinically as fever>99.5°F, myalgia, arthralgia, maculopapular rash, lymphadenopathy. Dengue fever is commonly known as "break bone fever", term coined by Benjamin Rush in 1780 after an epidemic in Philadelphia [2]. Also known as "Dandy fever" after slaves in West Indies who contracted dengue fever had change in posture and gait [3]. The known serotypes are DEN 1-4, and recent identified 5th serotype. Serious forms of dengue infection may present with haemorrhagic manifestations potentially leading to Dengue Shock Syndrome. The female Aedes mosquito usually becomes infected with the dengue virus when it takes a blood meal from a person during the acute febrile or viremic phase of illness. After an extrinsic incubation period of 8 to 10 days, the mosquito becomes infected. The virus is transmitted when infected mosquito bites and injects its saliva into person bitten. Dengue begins abruptly after an intrinsic incubation period of 4 to 7 d. There is also evidence of vertical transmission of dengue virus from female mosquito to the next generation. The WHO has provided guidelines in 2009 for efficient and accurate diagnosis of Dengue infection, gold standard for detection being virus isolation and identification, viral nucleic acid detection, serological tests for NS1 and IgM [4]. In this study, comprehensive use of NS1 antigen and IgM antibody ELISA was done in terms of usefulness and their applicability for virus detection and diagnosis in acute phase of illness.

MATERIALS AND METHODS

A prospective study for a period of one year from June 2023 to June 2024 was carried out on a total of 13531 blood samples received from inpatients aged between 03 to 45 y admitted in wards with complaints of fever>99.5°F, myalgia, arthralgia for more than 3 d duration. All the samples were received and processed in Virology Research and Diagnostic Laboratory, Department of Microbiology, Andhra Medical College, King George Hospital, Visakhapatnam.

Blood samples received were centrifuged @3000 rpm for 10 min, serum seperated and those single sera samples were tested for both NS1 antigen and IgM antibody by ELISA methodology. Merilisa *i* Dengue NS1 Ag and NIV IgM Capture ELISA kits were used for detection of NS1 Ag and IgM Ab in this study. OD value was calculated based on the cutoff value following kit insert instructions [5, 6]. Reading more than the cutoff was interpreted as positive or reactive. In this study, NS1 cutoff value was calculated by taking a mean value of added 3 NCs; and for IgM cutoff was 3 times the NC value. Other laboratory tests performed were complete blood count, liver function tests, serum creatinine, infection markers like CRP and Procalcitonin. Other febrile aetiologies proven for malaria, enteric fever, scrub typhus, chikungunya, COVID-19 were excluded in the study.

RESULTS

In this study, out of total 13531 samples processed, 2212 were positive (16.3%) both by NS1 and IgM ELISA. Male population, 1736 (78.5%) was more vulnerable than female population, 476 (21.5%). Maximum number of positive cases were recorded in September 2023 (20.2%) and October 2023 (15.2%) table 1. Fever was the commonest presentation (100%) table 2. Thrombocytopenia was seen in 84.3% of cases. Cutoff values for NS1 and IgM ELISA were 100 (mean of 3 NCs = 300 divided by 3) and 0.45 (NC × 3 = 0.15 × 3) respectively. 100% cases were detected by NS1 and 88% by IgM methods.

Table 1: Monthly distribution of dengue cases

Month	Total tested, n=13531	Total positive, n=2212	Percentage %
June 2023	874	113	5.1
July 2023	892	186	8.4
August 2023	1455	282	12.7
September 2023	1678	448	20.2
October 2023	1666	337	15.2
November 2023	1348	208	9.4
December 2023	996	112	5.1
January 2024	865	104	4.7
February 2024	695	98	4.4
March 2024	704	77	3.5
April 2024	717	83	3.7
May 2024	737	71	3.2
June 2024	904	93	4.2

Table 2: Clinical profile among dengue positive cases, n=2212

Signs/Symptoms	Total cases	Percentage %
Fever>99.5°F	2212	100
Headache	2168	98
Retro orbital pain	1742	78.7
Nausea	163	7.3
Vomiting	08	0.4
Myalgia	2114	95.6
Arthralgia	1849	83.6
Rash	118	5.3
Diarrhoea	14	0.6
Tachycardia	718	3.2
Bradycardia	1064	4.8

DISCUSSION

Dengue is an arthropod-borne viral illness transmitted by bite of daytime feeding female Aedes mosquitoes. In India, Aedes aegypti is the main vector in the most urban areas; however, Aedes albopictus is also incriminated in many states. Aedes polynesiensis and Aedes niveus have also been incriminated as secondary vectors in some countries. Dengue has been ranked as a rapidly emerging endemic disease. Dengue cases are seasonal, with maximum number of record seen during monsoon, which may be due to water stagnation, which becomes an excellent breeding site of mosquitoes. The climatic conditions, particularly temperature and rainfall, play key role on life cycle, breeding and longevity of vectors and, thus transmission of disease. Average survival of Aedes aegypti is 30 d and Aedes albopictus is 8 w [7]. During the rainy season, when vector survival is longer, the risk of transmission is greater. Early diagnosis is pertinent in forecasting endemic or epidemic warning [8]. Laboratory diagnostic tests like rapid diagnostic methods (RDT), ELISA, PCR are available for early detection; other procedures like viral isolation, complement fixation, haemagglutination inhibition, neutralization tests are available in higher settings [9].

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 virus, viral nucleic acid, viral antigen or antibody or combination of both these techniques. After the onset of illness, post onset of symptoms, the virus can be detected in human serum and plasma for 1 to 15 d. The choice of diagnostic methods depend on the purpose for which testing is done, the type of laboratory facilities and technical expertise available, costs, time of sample collection. No single laboratory test can be used to accurately diagnose disease over the course of illness [10]. Most widely used method for diagnosis dengue is RDT and ELISA. RDT are commercially available for testing anti-Dengue IgM, IgG antibodies and NS1 antigen which give result in 15 to 20 min. But, ELISA methodology is quite sensitive and specific when compared to RDT. In this study, ELISA was preferred to detect NS1 antigen and IgM antibody on single serum sample. During high viremia in initial days of illness, NS1 antigen levels can be identified as early as 5 d which disappear after appearance of specific antibodies.

The presence of secreted NS1 in the bloodstream stimulates a strong humoral immune response [11]. IgM can be detected at 3 to 4 d to as far as 90 d before declining. Anti Dengue virus IgM is produced during both primary and secondary infection. After 3 to 4 d of infection, IgM rises rapidly and is usually identified after 5 to 6 d. Its titre reaches peak at 14 d and declines after 90 d [12]. A positive NS1 antigen test result id indicative of dengue virus infection. A negative NS1 result does not exclude or rule out infection, in which case sample should be tested for presence of dengue IgM antibody to determine possible recent dengue exposure. A positive IgM in single sera sample are classified as presumptive, recent dengue viral infection [13].

The present study was undertaken to evaluate the utilization of ELISA technique in diagnosing dengue cases in acute phase of illness. Management of dengue fever is conservative; where in correlation with biochemical laboratory findings, especially platelet counts are to be monitored as it is the earliest prognostic marker [14].

In the present study, common clinical symptom for admission revealed fever>99.5°F for>3 d (100%), headache (98%), myalgia (95.6%). Male preponderance of 78.5% was recorded. Majority of cases in our study were positive in September 2023 (20.2%) and October 2023 (15.2%). Biochemical lab parameters like thrombocytopenia (84.3%), elevated AST (55%), leucopenia (48.7%), elevated ALT (47.4%) table 3 were also recorded. Serum Procalcitonin was raised in 100% of cases.

ELISA kits used in this study were Merilisa *i* Dengue NS1 antigen and NIV DENGUE IgM Capture ELISA; as screening and confirmatory tests as per WHO guidelines. The kits are intended to be used for *in vitro* qualitative detection of Dengue NS1 antigen and IgM antibody in human serum or plasma. Merilisa *i* Dengue NS1 antigen is a one step sandwich format microplate enzyme immunoassay. IgM ELISA assay involves capture of IgM antibodies in patients' serum by anti-human IgM (μ chain specific) coated on to the wells.

Cut off values were calculated as per the kit inserts, 100 for NS1 (mean value of 3 negative controls = 300/3) and 0.45 (0.15 × 3). Samples exceeding the reference cut off were read as reactive for NS1 and positive for IgM.

Table 3: Biochemical laboratory parameters in total dengue cases, n=2212

Parameter	Observation in total cases	Percentage %
Thrombocytopenia	1864	84.3
Leucopenia	1078	48.7
Lymphocytosis	819	37
Elevated Hematocrit	921	41.6
Elevated ALT	1049	47.4
Elevated AST	1216	55
Serum Creatinine	402	18.2
Elevated CRP	2182	98.6
Elevated Serum Procalcitonin	2212	100

Diagnostic sensitivity and specificity of Merilisa *i* Dengue NS1 antigen is 99.5% and 100%; and of NIV DENGUE IgM Capture ELISA is 98.53% and 98.84%. In our study, positive predictive value of NS1 was 100% and IgM was 88% table 4. Treatment modalities included oral antipyretics, intravenous fluids, symptom-specific supportive therapy and platelet transfusion with Single donor aphaeresis (SDP) was done in 12 cases with platelet count<60,000/ml of blood. Dengue has been identified as one of the 17 neglected tropical diseases by WHO in their first report on neglected tropical diseases (2010) [15]. The WHO has provided guidelines in 2009 for efficient and accurate diagnosis of dengue infection-Gold standard for dengue detection are viral isolation and identification, viral nucleic acid detection, serological tests for IgG, IgM, NS1.

NCVBDC (National Center for Vector Borne Diseases Control), Government of India has identified a netwok of laboratories fir surveillance of dengue fever cases across the country since 2007, free diagnostic facilities in endemic areas linked with 17 Apex Referral Laboratories (ARLs). Test kits are provided through NIV Pune since 2007 [16]. According to NCVBDC, in 2023 a total of 289235 cases with 485 recorded deaths were documented; and in 2024 till April 2024 recorded cases were 19447 with 19 deaths. No mortality recorded in this study.

Table 4: Positive predictive value of NS1 and IgM ELISA, n=2212

Parameter	Total positive	Total negative
NS1 Antigen	2212 (100%)	0
IgM ELISA	1946 (88%)	266 (12%)

CONCLUSION

In this study, comprehensive use of NS1 and IgM ELISA is proved as most appropriate method for detection of acute phase of dengue fever on a single serum sample without requirement of paired sera. In order to provide early public health control measures of dengue outbreaks, it is important to establish the diagnosis of acute dengue virus infection during first five days after onset of symptoms. The diagnosis is still a great challenge in developing countries like India, due to lack of resources, infrastructure, commitment and skilled manpower. Development of vaccine and significant clinical trials for safety and efficacy can reduce hospitalization and death in severe dengue cases.

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CONFLICTS OF INTERESTS

There are no conflicts of interest

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