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A cross-sectional study of epidemiology and determinants of Dengue positive cases reported at GGH, Mahbubnagar.

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ABSTRACT: Dengue fever is one of the common viral illnesses linked with significant morbidity and mortality. Oflate, there is rapid increase in dengue cases in India. The present study aimed to known dengue seropositivity indifferent seasons of a year 2023, and other laboratory parameters. Duringthe study period of 6 months 1072 patients hospitalized with probable dengue fever were included. Dengue serology was done for all cases by ELISA (J. Mitra& Co). Of the 1072 suspected dengue cases, 277 had confirmed dengue illness. Of these 277 cases, 78 showed Ns1antigen (28.16%), 199 IgM antibody (78.14%), and 74 had both Ns1 and Ig M (48.6%). Platelet count less than1,00,000 were seen in 266 (91.39%) patients. To minimize the load of dengue cases and its death rate better community awareness and vector control measures need to be strengthened during monsoon especially in areas where burden of the disease is more. This study helps in early preparedness of the authorities concerned in controlling possible epidemics in future.

Keywords: Dengue, ELISA, Ig M, NS1, Serology

I. INTRODUCTION

Dengue is an acute, potentially fatal viral infection that can culminate into dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). It is caused by four serotypes of dengue virus (DV), namely DEN-1, DEN-2, DEN-3 and DEN-4 belonging to genus Flavivirus and family Flaviviridae. It is spread through the bite of infected *Aedes aegypti* mosquito.[1] Most primary infections are uneventful. Infection with one serotype confers an individual life-long immunity to that serotype and cross-reactivity to the other serotypes. The complications like DHF and DSS are usually attributed to this cross-reactivity. [1,2] Dengue is almost endemic throughout India. The resource poor health care system has to depend upon simple to perform and easy to interpret laboratory tests for diagnosis. It is known that early and specific diagnosis of DHF or DSS followed by supportive therapy reduces morbidity and mortality.[3] The variability in the clinical illness associated with dengue infection (DI) cannot be accommodated in a single clinical definition. However, confirmation of DI is the most essential pre-requisite in the management of complications.[4]

The ‘gold standard’ tests for identification of DI are not within the reach of peripheral and even most tertiary carelaboratories. Detection of dengue specific IgM/IgG has been the mainstay of diagnosis of DI. Antibody detectionis an indirect method of diagnosis and, therefore, is prone to false positive as well as false negative results.[5] Of late,non-structural protein 1 (NS1) detection is available for diagnosis of DI.[6] NS1 detection is reported to be sensitiveas well as highly specific.[7] Apart from the dengue specific parameters, platelet count is the only accessory laboratorytest available in the peripheral areas that can support the diagnosis of DHF or DSS. Even in remote areas, plateletcounts can be roughly estimated by microscopy.[8]

Hence, the present study was carried out to evaluate the laboratory profile along with epidemiological pattern of dengue positives reported to GGH, Mahbubnagar, Telangana during the period June-December,2023.

II. Materials and Methods:

Type of study: Cross-sectional study
Duration: June-December,2023
Location: OPD,General medicine Dept andCentral Lab, GGH, Mahbubnagar, Telangana
Ethical consideration and permission: The study was approved by research and ethical committee ofthe institute.
Inclusion criteria: All the seropositive cases of dengue during the study period were included.
Exclusion criteria: Patients with fever but negative for dengue serological markers and patients with only IgG positive serology were excluded from the study.
Sampling method: Since our laboratory works round the clock, the samples were tested immediately for NS1, IgM and IgG by ICT-based tests. The test kits used were Advantage dengue NS1 Ag and Ab Combi Card supplied by J Mitra and Co. Pvt. Ltd, New Delhi, India The tests were performed strictly as per the manufacturer's instructions. NS1 antigen alone positivity indicates the early phase of illness.The concurrent NS1 antigen and IgM antibody positive status shows early phase of the illness. Triple positives indicate an early phase of a secondary infection. During the acute phase of the disease, the presence of IgM antibody alone indicates a primary infection. Based upon this, cases were divided into 3 serogroups i.e., NS1 positive,NS1+IgM positive, IgM positive.
For hematological analysis, EDTA sample was runin SYSMEX XN1000. Peripheral blood examinationand complete blood counts were done in all thecases and the following hematological parameterswere analyzed-Hemoglobin (Hb), Total Leucocyte count (TLC) and Platelet counts of all the cases positive for any of the dengue parameter were recorded. This evaluation has been done keeping in view thescenario at the peripheral centers, where only ICT-based tests are available for diagnosis of DI. No healthy controlswere included in the study as it has been amply proved that NS1 positivity is negligible in this group.

III. RESULTS

Table-1: Distribution of dengue seropositivity

Test (n=277)	Positive	%
IgM	199	71.84
NS1	78	28.16
Both	74	48.6

During the study period of 7 months (June -December 2022), of the total 1072 samples, 277 confirmed positive dengue illnessthrough ELISA. Of these 277 cases, 78 showed Ns1 antigen (71.4%), 199 IgM antibody (28.16%), and 74 had both Ns1 and Ig M (48.6%) as shown in (Table 1).

Table-2: Socio-Demographic profile of Dengue Positive patients

Age (yrs.)	N=277	%
<10	34	12.27
10-18	88	31.77
19-40	105	37.91
41-60	42	15.16
>60	8	0.03
Gender		
Male	135	48.74
Female	142	51.26
Residence		
Urban	65	23.46
Rural	212	76.54

Among 277 dengue positive cases, majority (37.91%) belongs to 19-40yrs age, while few were >60yrs age (0.03%) and 12.27% were below 10yrs age group. 48.74% were male and 51.26% were female. Majority were from rural areas, 76.54% and few are from urban areas,23.54%. (Table-2).Teenagers and young adults are most commonly affected as 69.68% cases were in the age group of 10-40 years. Least number of cases were seen in elderly (0.03%). There was female predominance (F:M- 1:1.1).

Table-3: Distribution of various Laboratory parameters

Lab Parameters	N=277	%
Total Leucocyte Count		
≤4,000	51	18.41
4000-11000	119	42.96
≥ 11,000	107	38.63
Platelet count		
≤10,000	13	0.05
10,00-1,00,000	253	91.34
≥ 1,00,000	11	8.63
Hemoglobin		
<10gm%	49	17.69
>10gm%	228	82.31

Evaluation of various hematological parameters (Hb%, Total Leucocyte counts, Platelet counts) is shown in Table 2&3.The blood profile of the 277 positive dengue illness patients showed decreased total leukocyte count (≤4,000) in 51(18.41%) patients,increased total leukocyte count (≥11,000) in 107 (38.63%) patients, increased (≥10gm%) Hemoglobin in 228 (82.31%) and platelet count less than 1,00,000 were seen in 266 (91.39%) patients as shown in (Table 3).

Table 4: Comparison of platelet counts with various permutations and combinations of dengue parameters

Parameter	Total	Platelet count <1,00,000/ml	%
NS1 only	78	77	98.71
IgM only	199	189	94.97
NS1 and IgM	74	71	95.94
Total	277	266	96.02

We tried to find the association of dengue parameter positivity with thrombocytopenia. The comparison of platelet counts with different dengue specific parameters is shown in Table 4. Of the 277 cases, 266 (96.02%) showedthrombocytopenia. In 78 cases that were positive for NS1, thrombocytopenia was evident in 77 (98.71%) cases.

In contrast, when only antibodies were considered for the diagnosis of DI, thrombocytopenia was noted in 189 of 199 (94.97%) cases only. Association of thrombocytopenia with NS1 was found to be higher by SEP test = 5.01, Z=3.51and *P* value <0.001, highly significant. Further analysis of two groups IgM only (94.97%) v/s NS1 plus IgM (95.94%) showed that thrombocytopenia was associated excellently when both NS1 and IgM were positive compared to IgM alone (SEP = 6.06, Z=3.37, *P*<0.001, highly significant). The role of antibody in the pathogenesis of dengue fever iswell-known. Therefore, better association of platelet count with detection of antibody is consistent.

IV. DISCUSSION

For a long time, detection of dengue-specific IgG/IgM has been the mainstay of diagnosis of DI. The dengue-specific antibodies begin to appear only around fifth day of fever in primary infection.[11] Even in most secondary infections, both the IgM and IgG type antibodies cannot be recorded before third day.[12] Therefore, there is always a window period, both in primary and secondary DI when only antibodies are tested. The new parameter, now available, for diagnosis of DI, the NS1 antigen, is detectable from day 1 of fever both in primary and secondary infections. It is important to note that NS1 is shown to be highly specific viral marker making it extremely reliable parameter for the diagnosis of DI from day 1 of the fever.[13]

In our study IgM antibody positives patients were more in number (71.84%) when compared to NS1Antigen positives and/or both NS1 andIgM positive patients. A similar study conducted by Rao[14] et al., 2021 (71.4%) and Mohamed[15]*et al.*, 2014 (76.7%), showed different results remarking significant difference in both the study.

Our study showed leukopenia in 18.41% patients and Leukocytosis in 38.63% patientswhereas in the study conducted by Rao et al., 2021[14] showed leukopenia in 39.29% patients and leukocytosis in 16.03% patients respectively and Katari*et al.*,2019 [(16))] showed leukopenia in 30.2% patients andleukocytosis in 8.1% patients respectively, whichshowed significant differences in both the studiesremarking that more number of patients in ourstudy are encountered with both leukopenia andleukocytosis.

In our study, platelet count ≤ 1, 00,000was seen in 91.39% patients but in the studyconducted by Rao et al., 2021[14] ,Katari*et al.*,2019 [(16)] and Adarsh andSubramanian, 2015 [(17)]platelet count ≤1,00,000was seen in 69.27% ,89.46% and 86.29% patientsrespectively, remarking that in our study a greater number of patients were encountered withthrombocytopenia.

Similarly in our study increased hemoglobin was seen in 82.31% patients but inthe study conducted by Rao et al., 2021[14], Katari*et al.*,2019 [(16)]Mohamed *et al.*, 2014[15] showed hemoglobin in 35.74% ,67.59% and 57.53% patientsrespectively showing significant differences in all the studiesremarking that greater number of patientsshowed raised hemoglobin in our study.

The correlation of dengue serology reports with platelet count in all patients is presented in Table-4. Dengue is usually accompanied with immune mediated destruction of platelet. The present study has also revealed a decrease in platelet count with positivity for IgM, NS1& both IgM and NS1 respectively.

V. Conclusion

Dengue is one of the emerging infectious disease in the recent years . Our study highlights the clinical pattern of presentation of the disease in correlation with the laboratory parameters and the disease outcomes. It can be concluded that the clinical presentation of dengue illness varies considerably from place to place. In order to minimize the load of dengue cases and its death rate, increased community awareness and vector control measures need to be strengthened during monsoon period, especially in Rural areas Mahbubnagar district.

This study has been carried out at a tertiary care teaching hospital. It is worth mentioning here that most tertiary care teaching hospitals lack in viral culture setup. Therefore, applying gold standard tests in studies related to viral infections is out of reach of these centers. Dengue is an infection that is present in urban, semi-urban, and rural areas. Our healthcare system is extremely resource poor. Top class technological backup is available only at very few elite laboratories situated in big cities. It is important to conduct studies in the peripheral centers where the laboratory must function without great technological backup and still is expected to provide reasonable opinion to the clinician in the management of infections like dengue.

The limitation of the present study was that Polymerase Chain Reaction (PCR) could not be used.[12] Most of the cases comingto our hospital were referred from various places and had received a few days treatment before reaching this hospital.The precise day of fever at the time of conducting the test could not be obtained in many cases. Despite this, NS1 only was positive in 28.16% cases. Given an opportunity to test every case of fever on day 1, a greater number of cases could have been picked up by NS1. It is shown that the titers of NS1 represent the viral load and the viral load is directly proportional to complications.[9] It can be logically inferred that in complication prone cases, i.e. having higher viral load, detection of NS1 will be easier because of higher NS1 levels. This would reduce the chances of false negativity by a less sensitive test like ICT. We therefore feel that inclusion of NS1 in the test panel either in micro-ELISA or ICT test format must be includedfor evaluation of all cases of fever, either in endemic or non-endemic areas. The ease, speed and dependabilityof ICT make it an excellent tool in addressing this potentially fatal, epidemic prone infection that has becomean important public health problem in our country. One can never forget the fact that dengue often breaks out inresource poor peripheral areas where ICT-based tests could be the only support available.

CONFLICTS OF INTEREST:

FUNDING

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