Retrospective Study of Optimization of Various Available Laboratory Interventions at Peripheral Centre in Management of Dengue Cases

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Abstract

Dengue Fever is a mosquito-borne illness commonly present throughout the tropics. Dengue virus belongs to flaviviridae family and circulates with 4 different serotypes circulating in environment. In India four different serotypes of the virus are seen throughout country. The disease has a wide spectrum from an asymptomatic stage to DHF and DSS. Annually 100 million cases of dengue and a half million cases of DHF occurs worldwide with a case fatality of 0.5% to 3.5%. The combined laboratory interventions with the clinical spectrum can reduce the mortality in disease. With this background study was carried out at a peripheral centre to optimise, correlate and predict the laboratory interventions in management of the patients. Probable cases of dengue fever were selected clinically in both indoor and outdoor patients. Retrospective study was carried out at a peripheral centre for duration of 06 months. Depending upon the immunopathogenesis of the disease different tests were combined. Composite haematological, rapid and serological methods were used in all the patients. All the parameters in the data sheet were scrutinised in detail. MacElisa is a highly specific test for the diagnosis of dengue so it was used for the selected cases. Total no of 1158 samples were collected from patients. Major cases reported during August to September. 16.75% were NS1 positive, 6.3% IgG positive and 3.8% IgM positive. Mac ELISA detected IgM antibody in 2.15%. There was decrease in platelet count in more no of patients because of decrease in sensitivity or simultaneous progression of some other viral illness. Haematological parameters helped in monitoring progression of illness. Though most of the dengue cases are asymptomatic or which may recover after a fever spike. There have been several reports of panic situation in community because of asymptomatic cases. Combined investigation approach would definitely reduce the panic condition, psychological effects and would reduce the complications of the disease if any. Early detection of NS1 the antigen would help in monitoring of cases and regular surveillance with haematological parameters. Rapid methods are easy to perform, interpret and less time consuming. Secondary antibody IgG was present in 12.34%. Secondary antibodies can facilitate the progression of the disease to DHF or DSS. Secondary antibody would help in understanding the progress of disease spectrum. Further to add to the study Mac ELISA was performed. At peripheral centres there are no facilities for the blood component preparation and storage. Dengue is serious with a low platelet count. Considering these things at a peripheral centre, combined approach of clinical and laboratory methods were used. These methods are appropriate for timely evacuation of the patients with reduction in mortality and morbidity. In the present study also one patient progressed to DHF. He was immediately transferred to tertiary care centre and successfully managed. Thus we recommend for the combined approach in periphery for better clinical outcome.
Introduction

The word dengue is derived from Swahili ‘ka-Dinga pepo’ which literally means cramp like seizure from an evil spirit (Rush, 1789). The exact origin of the disease is in question. Dengue Fever is a mosquito-borne illness commonly present throughout the tropics. Dengue virus is single stranded RNA virus belongs to flaviviridae family (Guzman et al., 2002). There are 4 different serotypes of the dengue virus. There is an epidemiological characterisation of various geographic regions depending upon the presence of single or multiple serotypes. In India four different serotypes of the virus are seen throughout country. The disease has got a wide spectrum from an asymptomatic stage to DHF and DSS. Annually 100 million cases of dengue and a half million cases of DHF occurs worldwide with a case fatality of 0.5% to 3.5% (Halstead, 1999). The combined laboratory interventions with the clinical spectrum can reduce the mortality in disease. With this background study was carried out at a peripheral centre to optimise, correlate and predict the laboratory interventions in management of the patients.

Materials and Methods

Study population

Probable cases of dengue fever were selected clinically in both indoor and outdoor patients. Different types of age group and demographic features were selected in the study. The details of other co-morbidities were also noted.

Inclusion criteria

Any fever case whose underlying condition is not diagnosed or proved or the probable cases of dengue (https://wwwn.cdc.gov/nndss/conditions/dengue-virus-infections/case-definition/2010/).

Study design

Retrospective study was carried out at a peripheral centre for duration of 06 months. The demographic details were recorded in detail. Samples were processed in the laboratory after the clinical assessment from the indoor and outdoor patients. Follow up of the patients was done with suitable laboratory parameters.

Laboratory parameters

Depending upon the immunopathogenesis of the disease different tests were combined. Composite haematological, rapid and serological methods were used in all the patients. Haematological parameters included monitoring of HB, TLC, DLC, PCV, Platelet count and PBS examination. Rapid methods were based on Immunochromatography principle with an ability to detect the test within 30 minutes. Rapid kits were used for diagnosis of Non structural Antigen (NS1-Ag) and Antibodies against dengue infection like IgM and IgG. Serological methods were based on 4th generation ELISA to detect IgM.

Protocol of the procedure

Blood samples were collected in EDTA and plain vacutainer with all standard aseptic measures. Haematological parameters were processed by an automated analyser and analysed the results subsequently. Manually peripheral blood smears (PBSs) for each samples were also prepared by standard method and examined subsequently. Serum samples were used for both the Rapid serological tests and MacElisa tests. All the parameters in the data sheet were scrutinised in detail.

Haematological tests were processed for all the fever cases during 1-3 days of his illness. Rapid serological tests were processed for febrile illness of 3 -7 days. Meanwhile both
the parameters were correlated. Rapid serological tests were repeated in some patients with antigenemia without development of primary antibody. Haematological parameters of those patients with antibodies were monitored daily.

MacElisa is a highly specific test for the diagnosis of dengue. Therefore it was used for the selected cases as

1. Abnormal platelet count
2. Positive primary or secondary antibody
3. Presence of petechie, rash or red colored urine

**Results and Discussion**

Total no of 1158 samples were collected from patients out of which 886 were from opd and 272 were from ipd. All these samples were processed for haematological and rapid tests.

Dengue is a mosquito borne viral infection with increasing distribution in tropical countries. Though most of the cases are asymptomatic and which may recover after a fever spike. This Dengue fever cases although normal may cause a panic situation in community. Anurag jhanjee carried out study at delhi to see the psychological manifestation of dengue fever. In this study fraction of people had psychological manifestation (Jhanjee et al., 2013). Combined investigation approach would definitely reduce the panic condition, psychological effects and would reduce the complications of the disease if any.

Dengue NS1 antigen is a highly conserved nonstructural glycoprotein (46-kDa ), exists as an intracellular, membrane-associated and as an extracellular form secreted from DENV-infected mammalian cells (Winkler et al., 1989). The membrane associated form is essential for the viral replication. Extra cellular or the cell surface associated form is involved in signal transduction (Sophie et al., 2005). Therefore NS1 Ag was tested for all the febrile cases. In the present study NS1 positive cases were 73 (24.34%).

| Month | No of Rapid tests | NS1 +ve | IgG +VE | IgM +VE | Mac ELISA total tests | Mac ELISA +ve |
|-------|------------------|--------|--------|--------|-----------------------|---------------|
| Aug   | 263              | 38     | 15     | 05     | ---                   | ---           |
| Sep   | 184              | 47     | 04     | 05     | 32                    | 09            |
| Oct   | 252              | 63     | 30     | 23     | 50                    | 12            |
| Nov   | 167              | 42     | 18     | 09     | 47                    | 02            |
| Dec   | 20               | 04     | 06     | 02     | 10                    | 02            |

As per Table-1 major cases reported in August to September. Out of these samples 16.75% were positive for NS1 detection, 6.3% for IgG and 3.8% for IgM. Mac ELISA detected IgM antibody in 2.15%.

| Decrease in platelet count | NS1 Positive | IgM Ab Positive | IgG Ab Positive |
|---------------------------|--------------|-----------------|-----------------|
| 284 (24.52%)              | 194 (16.75%) | 44 (3.8%)       | 73 (6.3%)       |

There was decrease in platelet count in more no of patients because of decrease in sensitivity or simultaneous progression of some other viral illness. Haematological parameters helped in monitoring progression of illness.

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Early detection of the antigen would help in monitoring of cases and regular surveillance by haematological parameters.

Rapid immunochromatography methods are easy to perform, interpret and less time consuming. IgM is the primary antibody which may appear as early as within a wk. in the present study also 16% samples were positive for IgM at a single point of intervention. The samples were tested within a wk for the antigen and IgM. The other immunoglobulin IgG is a secondary antibody. This secondary antibody was also present in 12.34%. Presence of secondary antibodies can facilitate the progression of the disease to DHF or DSS. Thus presence of primary or secondary antibody would help in understanding the progress of disease spectrum.

Further to add to the study Mac ELISA was performed. These methods identify the probable dengue cases and helps in surveillance. There have been reports of app 50% positivity of IgM. In the present study these were less to app 12.34%, the other studies had a very high rate of IgM

At peripheral centres there are no facility for the blood component preparation and storage. Dengue is serious with a low platelet count. Considering these things at a peripheral centre combined approach of clinical and laboratory method was used. These methods are appropriate for timely evacuation of the patients with reduction in mortality and morbidity. In the present study also one patient progressed to DHF. He was immediately transferred to tertiary care centre and successfully managed. Thus we recommend for the combined approach in periphery for better clinical outcome.

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