Original Research Article

Serodiagnosis of dengue viral infection with platelet count correlation in patients presenting to a tertiary care hospital

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Abstract

Background: Dengue viral infection is an arboviral infection transmitted by Aedes mosquito more common in children than adults is widely distributed in India can cause serious complications such as Dengue haemorrhagic fever(DHF)&dengue shock syndrome(DSS) there is no effective vaccination for dengue fever early detection is important in the management of dengue fever. **Material and Methods:** A retrospective study of Dengue serology and platelet count of patients blood samples collected in central lab from 1 January 2018 to 31 December 2019 was done at Medi Citi Institute of Medical sciences Medchal Telangana. **Results:** Of the 753 patient samples tested 262 were tested Positive for either NS1, IgM and IgG, of the 262 samples NS1 positive only were 138 (52.67 %) followed by IgM positive only 57 (21.75 %), IgG positive only 5 (1.90%) NS1 and IgM positive only 56 (21.37%), NS1 and IgG positive only 1 (0.38%), IgG and IgM positive only 5 (1.90%). Platelet count less than 1 lakh was seen in 140 patients, In NS1 positive patients 67 (48.55%) had thrombocytopenia followed by IgM only positive patients 40 (70.18%) IgG only positive 5 (100%), NS1 and IgM positive only 22 (39.29%), NS1 and IgG positive only 1 (100%) and IgG and IgM positive only 5 (100%) had thrombocytopenia. **Conclusion:** NS1 antigen remains important in the detection of dengue viral infection as maximum number of dengue fever in this study were detected by NS1 antigen and largest group of patients having thrombocytopenia were associated with NS1 antigen detection Out of 195 total NS1 positive cases thrombocytopenia was seen in 90 patients.
Key Word: dengue, platelet count.

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INTRODUCTION

Dengue is an acute viral infection, endemic in 35 states and union territories of India. Dengue virus belongs to genus Flavivirus in the family Flaviviridae is a single stranded enveloped RNA virus, Four serotypes of dengue virus exists DEN1, DEN2, DEN3 andDEN4 infection with one serotype confers lifelong immunity to that serotype and cross reactivity with other serotypes Dengue is transmitted from human to human by mosquito bite, Man is the reservoir of the virus, Aedes aegypti is the most efficient of the mosquito vectors, Dengue viral infection can range from mild fever to severe haemorrhagic fever(DHF)and dengue shock syndrome(DSS). Dengue fever is more prevalent in paediatric age group Lab diagnosis of dengue viral infection depends on the diagnosis of dengue specific IgM, IgG and Dengue NS1 Antigen by rapid immunochromatographic methods, qualitative and quantitative methods such as CAPTURE ELISA and Polymerase chain reaction are more sensitive in the detection of dengue fever in the present study immunochromatographic method is used in the sero diagnosis of dengue also platelet count is done in3part haematology machine NS1 antigen detection in dengue starts from the first day of infection and hence it is

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important marker in serodiagnosis of dengue and also there is no need to repeat the test if it is positive hence dengue NS1 detection is the main stay of study also early detection of thrombocytopenia is important for managing dengue haemorrhagic fever and dengue shock syndrome as mortality is high with these complications

Aims and Objective: Detection of dengue viral infection among febrile patients Correlate platelet count in dengue positive patients for detection of thrombocytopenia

MATERIALS AND METHODS

We carried out retrospective study of Dengue serology and platelet count of patients from 1 January 2018 to 31 December 2019 both outpatients and inpatients blood samples of patients were collected in central lab were tested for Dengue IgM, IgG and NS1 antigen by rapid immunochromatographic method (ABBOTT), platelet count estimation was done by Avantor 3-part Haematology Analyzer

Inclusion criteria- Acute febrile illness with two or more manifestations headache, retro orbital pain, arthralgia, rash, haemorrhagic shock manifestations, leucopoenia and positive serologic tests

Exclusion criteria- A febrile patients with arthralgia, headache, rash and leucopoenia,

Since our lab runs round the clock samples were tested immediately for NS1, IgG, and IgM by immunochromatography tests by SD Bio line kits (ABBOTT) tests were conducted according to manufacturer instructions platelet count of all dengue positive and also dengue negative patients were recorded

Procedure

Sample collection- venous blood was collected from cubital vein after cleaning cubital fossa in the forearm with beta dine and 70% alcohol later blood is drawn into vacutainers by 22 gauge needle into both EDTA tubeand plain tube about 3 ml in each vacutainer.

Blood in plain vacutainer is kept aside for 30 minutes and centrifuged at 3500 rpm for 10 minutes the supernatant serum is used for immunochromatography tests 100 micro litres of serum is added towards NS1 antigen well, 10 micro litres of serum towards IgG/IgM well and 4 drops of buffer is also added in separate well the main principle of test is lateral flow immunochromatography if the tests are positive there is antigen antibody reaction with the formation of both test and control lines, if the test is negative only control lines are formed, if there is no control line the test is invalid each test is read after time of 15 minutes to say if it is negative or positive

Blood in EDTA vacutainer is kept on roller for 10 minutes and processed in Avantor 3part haematology machine the main principle of this machine is flow cytometry where cell size and shape are measured and report is given, in the present study platelet count is taken into consideration, count of less than 1 lakh/per micro litre of blood is taken as thrombocytopenia

RESULTS

Of the 753 patient samples tested 262 were tested Positive for either NS1, IgM and IgG, of the 262 samples NS1 positive only were 138 (52.67 %) followed by IgM positive only 57 (21.75 %), IgG positive only 5 (1.90%) NS1 and IgM positive only 56 (21.37%), NS1 and IgG positive only 1 (0.38%), IgG and IgM positive only 5 (1.90%) Platelet count less than 1 lakh was seen in 140 patients, In NS1 positive patients 67 (47.86%) had thrombocytopenia followed by IgM only positive patients 40 (28.571%),IgG only positive 2 (3.71%), NS1 and IgM positive only 22 (15.71%), NS1 and IgG positive only 1 (0.71%) and IgG and IgM positive only 5 (3.57%) had thrombocytopenia.

Table 1: Comparison of dengue parameters

Parameter	Number	Percentage
NS1 Only	138	52.67
IgM Only	57	21.75
IgG Only	5	1.90
NS1 and IgM only	56	21.37
NS1 and IgG Only	1	0.38
IgG and IgM only	5	1.90
Total	**262**	**100.0**

IgM: Immunoglobulin M, IgG: Immunoglobulin G, NS1: Non-structural protein 1

Table 2: Comparison of Dengue parameters with < 1,00,000 Platelet count

Parameter	Total	< 1,00,000 Platelet	Percentage
NS1 Only	138	67	48.55
IgM Only	57	40	70.18
IgG Only	5	5	100
NS1 and IgM only	56	22	39.29
NS1 and IgG Only	1	1	100
IgG and IgM only	5	5	100
Total	**262**	**140**	

DISCUSSION

Since NS1 Antigen is detected from day one onwards of fever both in primary and secondary infections it is extremely reliable parameter IgM antibodies appear from the 5th day onwards ,hence there is a window period were the diagnosis of Dengue is missed if NS1 is not tested The distribution of various Dengue specific parameters is shown in table 1 of the 262 Dengue positive patients NS1 was detected in 138 (52.87%) that is more than half of the Dengue positive patients IgM positive 57 (21.84%) patients was the second most common followed by NS1 and IgM detection 56 (21.46%) where as studies by R.D.Kulkarni in 2011 IgM was the most common Dengue parameter 50% detected among 320 dengue positive patients, IgM indicates recent infection were as IgG may persist for several years, IgM also detected in secondary dengue infections, when NS1 is positive secondary testing is not required Regarding the association of Dengue parameters with thrombocytopenia in NS1only detected patients thrombocytopenia was 48.55% were as in IgM only detected patients it was 70.18%, NS1 and IgM only it is 39.29%, here also NS1 was important in detection of dengue fever associated with thrombocytopenia as 67 out 138 patients had trombocytopenia were as in R.D Kulkarni 2011 study in IgM positive cases 59.6 % was associated with thrombocytopenia Out of 195 total NS1 positive cases thrombocytopenia was seen in 90 patients, Z value was 1.7 and P value < 0.05 is significant also in Dengue only NS1 detected 138 cases thrombocytopenia was seen in 67 patients Z value was 3.6 and P value <0.05 is significant, in 491 cases of fever were all Dengue parameters were negative, 57 patients had thrombocytopenia the association of thrombocytopenia in dengue parameter positive cases was 140 Z=8.18 and P < 0.05 was significant The limitation of the present study was that CAPTURE ELISA Qualitative or Quantitative, Polymerase chain reaction (PCR) could not be used, CAPTURE ELISA has higher sensitivity than ICT based tests inclusion of CAPTURE ELISA in this study could have given comparative sensitivity of ICT based tests It is shown that Titres of NS1 represent viral load and viral load is proportional to complication despite CAPTURE ELISA and PCR virus isolation is gold standards in detection of Dengue viral infection but only referral labs have such facility and are absent in diagnostic and in many teaching institutions platelet count in Dengue viral infection is important as thrombocytopenia is common in Dengue fever leading to dengue haemorrhagic fever and dengue shock syndrome which carry high fatality rate Due to very large distribution of Dengue viral infection in India early detection of dengue viral infection and thrombocytopenia in dengue viral infections helps in better management of dengue haemorrhagic fever and dengue shock syndrome Immunochromatographic tests are easy to use and helps in early detection of dengue fever

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