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Original Research Article

Serodiagnosis of dengue using NS1 antigen, dengue IgM, dengue IgG antibody with correlation of platelet counts

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Abstract

Introduction: The dengue virus causes one of the most important mosquito-borne viral diseases.Annually, it affects up to 100 million people. Early laboratory diagnosis of dengue infection isimportant, as itcan allow early intervention and better prognosis. Dengue IgM antibody isdetected usually after five days of infection. NS1 antigen can be detected as early as day one.Platelet count is the only non-dengue parameter that can support the diagnosis of the dengueshock syndrome (DSS) and dengue hemorrhagic fever (DHF). This study was done to correlate the platelet count and dengue parameters detected either by rapid card test or by ELISA methods. **Materials and Methods:** Five hundred ninety three clinical samples suspicious of dengue were collected from April2020 to September2020. The received samples were tested by rapid methods and ELISA for dengue parameters and for platelet count by automated Becmon Coulter5partcellanalyser. **Results:** Out of 593 samples, 90 samples were positive by rapid tests that included NS1 antigentest, IgM and IgG dengue by immune chromatographic tests. Sixty one samples were positive bydengue IgM and dengue IgG ELISA.Samplespositivefordengue IgM antibodies orNS1antigen alone or positive for both were classified as primary dengue infection. Samples positivefor dengue IgG antibody or dengue IgG and IgM antibodies were classified as secondary dengue infections. Of the 90 cases positive by rapid card test, 62 cases were classified as primary dengueinfection and 28 cases as secondary dengue infection. Total 61 cases were positive by ELISAtests of these 35 cases were diagnosed as primary dengue and remaining 26 cases as secondary dengue infection. **Conclusion:** Thus the rapid tests such as NS1 antigen test, IgM and IgG dengue antibody can be used for the diagnosis in small peripheral laboratories. Thrombocytopenia was more consistent with cases positive for only IgG. Though MAC ELISA is more sensitive and specific than rapid methods, detection of NS1 antigen by immunochromatograpy test helps in early detection.

Keywords: Dengue; Dengue IgG; Dengue IgM; NS1 antigen; Platelet count.

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Introduction

Dengue is caused by a positive stranded RNA virus of the flaviviridae family with four distinctserotypes (dengue 1 to 4) that are related antigenically[1]. India is endemic for dengue infection.As of 2010 dengue fever is believed to infect 50 to 100 million people worldwide a year, withhalf a million life threatening infection. It has dramatically increased in frequency between 1960and 2010 by 30 fold. The geographical distributions around the equator with 70% of total 2.5billion people living in endemic areas from Asia and the Pacific[2]. To reduce the high mortalityrate and the disease burden, it is imperative to have a rapid and sensitive laboratory assay forearly detection of the disease. The diagnostic methods currently available are viral culture, viral RNA detection by reverse transcriptase PCR(RT-PCR) and serological tests such as animmuno globulin M(IgM) capture enzyme-linked immune sorbent assay(MAC-ELISA).However, early dengue diagnosis still remains a problem, as all these assays have their ownpitfalls. The first two assays have the scope as a routine diagnostic procedure. Viral isolation bycell culture and subsequent detection by immunofluorescence, though the gold standard, cannotbe used as a routine diagnostic procedure due to its low sensitivity, laborious procedure and time consumption[3,4,5]. The requirement of a highly trained staff, the need of a sophisticated equipmentas well as the cost factor associated with molecular methods has limited its application as aroutine diagnostic assay. Anti dengue IgM antibody appear as early as 3 days after infection andremain in circulation for one to two months.

Anti dengue IgG antibody appear after one week, peaks after 2 to 3 weeks and remain lifelong in circulation.2 The MAC-ELISA, which is acommonly used assay, has a low sensitivity in the first four days of illness[6]. The requirement of paired sera at acute and convalescent phase, which improves the accuracy of the test but furtherdelaysit. NS1 (non-structural protein 1)is a highly conserved glycoprotein thatis essential forthe viability of dengue virus andis produced both in membrane-associated and secretory forms by the virus[7]. The detection of secretory NS1 protein represents a new approach to the diagnosisof acute dengue infection. The wide variation of platelet counts is a natural process. Since themechanism of dengue related thrombocytopenia and coagulopathy is complex, it would involveplatelet activation, procoagulant and anticoagulant arms of the coagulation system, complement, cytokines, and endothelial cells. Moreover, asymptomatic thrombocytopenia would require platelet transfusion though platelet counts might not correlate well with clinical bleeding indicating that other features such as disturbance in platelet function and capillary fragility, contribute to the bleeding diathesis[11].

Material and methods

Five hundred ninety three clinical samples suspicious of dengue were collected from April 2020to September 2020 at our institution in Tagore Medical College and Hospital, Chennai.Allserum samples were tested by rapid card test and ELISA methods, and platelet counts werecorrelated for any of the dengue parameter. In rapid solid phase immunochromatograpy for the qualitative detection of dengue NS1 antigen, differential detection of IgG and IgM antibodies to dengue virus from patients was done by card methods. SD Bioline card test was used. Similarly all the serum samples were tested for dengue IgM, and dengue IgG antibodies by ELISA method. For dengue IgM ELISA, serum samples were tested by Pan Bio dengue IgM capture

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ELISA. This enzyme linked immunoassay detection of IgM antibody to dengue was done as permanufacturer's instructions. For dengue IgG ELISA serum samples were tested by Pan Biodengue IgG capture ELISA test. This enzyme linked immunoassay detection of IgG antibody todengue was done as permanufacturer's instructions. The permutations and combinations ofdengue specific parameters were correlated with thrombocytopenia. Platelet count was done by automated Becman Coulter 5 part cell analyzer. Platelet counts of all the cases positive for any ofthe dengueparameterwererecorded.

Results

The received samples were tested by rapid methods and ELISA, in which 90 samples werepositive by rapid tests that included NS1 antigen immune chromatographic test, IgM and IgG dengue immune chromatographic tests. Sixty one samples were positive by dengue IgM anddengue IgG ELISA. Table 1illustrates the serological positive cases by rapidmethod, ELISAand by both the methods. As illustrated in the Table 2, in rapid methods 23.3% samples werepositive for NS1 antigen immuno-chromatograpy test, 33.3% samples were positive by dengue IgM immune chromatograpy method, 12.2% were positive by both dengue IgM immune chromatograpy method and NS1 antigen. 16.7% were found to be positive for dengue IgG immune chromatograpy test.1.1%were positiveforNS1antigen,dengue IgM andIgGantibody altogether. 12.2% were positive for dengue IgM and IgG antibodies. Samples positivefor NS1 antigen, dengue IgM antibodies or for both NS1 antigen and dengue IgM antibody were classified as primary dengue infection. Samples positive for dengue IgG antibody or both dengueIgG and IgM antibodies were classified as secondary dengue infections. The rapid card testincludes detection of NS1 antigen, dengue IgM and IgG antibodies. Of the 90 cases positive byrapid card test 62 cases were classified as primary dengue infection and 28 cases as secondarydengue infection. All who had a platelet count of <100000/ul (as per WHO cut off for plateletcount for DHF) were considered as thrombocytopaenic. Of 21 cases positive for NS1 antigen, 15(71.4%) showed thrombocytopenia. Platelet counts decreased more in secondary dengue infections, of 15positive for only dengue IgG antibodies 13(86.6%) showed decreased plateletcounts. Table 3 depicts number and percentage of platelet count in relation to positive primaryand secondary dengue cases. As illustrated in Table 4, 35 cases were positive by dengue IgMELISA tests, 17 cases positive by dengue IgG ELISA, 9 cases positive by both IgG and IgMELISA test. Table 5 depicts ELISA tests positive in primary and secondary infections in relationto platelet count. 61 cases were positive by ELISA tests. Thirty five cases were classified as primary dengue and remaining 26cases as secondary dengue infection. In primary dengue infections 23(65%) cases showed thrombocytopenia. Platelet counts were more decreased in secondary dengue infections that is14(82.4%) cases showed thrombocytopenia out of 17cases.

Table1: Serologically positive cases by rapid, ELISA and by both the methods

Rapid test only	ELISA test only	Positive for both rapid and ELISA	Total no of positive cases
90	61	108	151

Table2: Serologically positive samples using rapid test methods

Rapid Test	Positive	%
NS1antigentest	21	23.3%
IgM	30	33.3%
NS1+IgM	11	12.2%
IgG	15	16.7%
NS1+IgG	1	1.1%
NS1+IgM+IgG	1	1.1%
IgM +IgG	11	12.2%
Total	90	100%

Table 3: Number and percentage of platelet count in relation to positive primary and secondary dengue cases

Rapid Test	No of Primary cases	No of Secondary cases	No of samples Platelet count <1lakh
NS1antigentest	21	0	15(71.4%)
IgM	30	0	17(56.6%)
NS1+IgM	11	0	11(100%)
IgG	0	15	13(86.6%)
NS1+IgG	0	1	1(100%)
NS1+ IgM+ IgG	0	1	1(100%)
IgM+ IgG	0	11	7(63.6%)
Total	62	28	--

Table4: Serologically positive cases diagnosed by ELISA test

Name of the test	No. of cases positive	%
IgM ELISA	35	57.4%
IgG ELISA	17	27.9%
IgM +IgG ELISA	9	14.7%
Total	61	100%

Table5: ELISA tests positive in primary and secondary infections in relation to platelet count

Name of the test	Primary Dengue infection	Secondary Dengue infection	No of cases showing platelet count<1lakh	%
IgMELISA	35	0	23	65.7%
IgGELISA	0	17	14	82.4%
IgM +IgGELISA	0	9	7	77.7%
Total	35	26	--	--

Discussion

Incidence of dengue has increased 30 fold with an expanded geographic distribution of both the viruses and the mosquito vector to new countries and from urban to rural settings[2].

The new challenge is recurrence of dengue haemorrhagic fever with higher severity, even twenty years after the primary infection. Dengue vaccine development has been in progress for several decade show ever the complex pathology of illness, the need to control 4virus serotype shampered progress of vaccines. In patients it gives new dimension to this disease and reinforces the need to get the long lasting immunity to all 4serotypes of viruses.

The vaccines should provide protective to four serotypes to avoid the ADE (antibody dependent enhancement) phenomenon[1]. The present study was carried out from April 2012 to September2012.

In 593 clinically suspected cases of dengue 151 were positive for dengue virus infection. Allthese samples were tested by rapid card test and ELISA test. All the positive samples for any ofthe dengue parameter were assessed for platelet counts. In 593 suspected cases for dengue 90cases came positive by rapid immune chromatographic test and 61 cases positive by ELISA method. Out of 90 cases, 62 were primary dengue infection of which 21(23.3%) were positive for NS1 antigen, 30(33.3%) were positive for IgM antibodies, and 11(12.2%) were positive for both NS1 antigen and IgM antibodies. This was similar to study by Sangetal[8]. Thrombocytopenia was more consistent with NS1 positive cases that is 71.4%. Similar results were seen in the study by Kukarani et al[2]. Similarly rapid Immuno chromatographic test detected a total of 28 cases as secondary dengue infection. Of these 15(86.6%) were positive for dengueIgG, 11(63.6%) were positive for both dengue IgG and IgM and only one case each positive fordengue NS1 and IgG and NS1, IgM and IgG. Hence, number of cases would be missed ifNS1antigen test was not included. In the present study all the samples were again tested with PANBIO capture ELISA. 61 were positive by this method, of these 35 were primary dengue infection, 26 were secondary dengue infection. Thrombocytopenia was more consistent withcases positive for only IgG that is 82.4%. Total 52 cases were positive for IgM and IgG byELISA.

Total of 45 were positive for IgM and IgG by rapid immune chromatograpy. So total of 7 cases were missed by rapid methods. Thus ELISA is more sensitive than rapid card test. Rapid card test could detect more case of primary dengue infection because it also Included NS1 antigendetection while the ELISA used in the current study did not include NS1 antigen. Titer of NS1represents the viral load and the load is directly proportional to complications. It can be logicallyinferred that in complication prone cases i.e. having viral load, detection of NS1 will be easier.The ease, time taken by the immune chromatography test makes it as an important method in peripheral areas where the laboratory has to function with poor facilities and provide the diagnosis to clinicians in the management of infections like dengue.

Conclusion

In the presentstudy 593clinically suspected denguefever cases were tested for NS1 antigen, IgM and IgG antibodies by immuno-chromatograpy methods. IgM ELISA and IgG ELISA methods were also done. In 593 suspected cases tested positive for dengue infection. Rapid cardtest detected total of 90 cases in which 62 were diagnosed as primary dengue infection of which 21 cases were positive for NS1 antigen, 31 werepositive for IgM, 11 were positive for IgM andNS1 antigen. ELISA detected 61 cases in which 35 cases were primary dengue infection. The ease and the time taken makes immune chromatography test for diagnosis of dengue an excellent tool in peripheral areas where laboratory has to function without great technological backup andstill is expected to provide reasonable opinion to the clinician in the management of infectionslike dengue. Inclusion of NS1 antigen in the immune chromatograpy further enhances the clinical utility immune chromatograpy. ELISA can be very effective in the diagnosis of dengue infection but limitations of the test include their inability to identify dengue virus type and potential crossreactivity with other flaviviruses. However, the need of the hour is in long term vector controls strategy so that outbreak can be prevented and this will simultaneously solve the problem of other mosquito borne disease like chickungunya, japaneseencephalitis, malaria, and filaria.

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