Correlation of serological markers with haematological parameters in early diagnosis of dengue infection in dengue prone areas

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Abstract

Background: Dengue is a serious, rapidly spreading tropical mosquito-borne viral disease caused by dengue virus belonging to the family flaviviridae and genus flavivirus in the last five decades. In many dengue endemic remote areas with limited laboratory diagnostic services their rapid diagnostic tests are simple method which provides the immediate diagnosis and early management. **Materials and methods:** The study includes clinically suspected 1236 cases with all haematological parameters and tested at clinical laboratory in which 500 serum samples showed positivity for one or more markers like NS1, IgM or IgG antibodies. **Results:** In our study among 500 seropositive cases thrombocytopenia (<1 lakh) was evident in 66.7% and in 736 seronegative cases thrombocytopenia was seen in 27.47%. Out of 323 NS1 positive cases, thrombocytopenia was evident in 56.0% and in exclusive antibody positive cases thrombocytopenia was noted in 19.2%. These associations were considered to be statistically significant. **Conclusion:** The problem of dengue is complex in India because large population, poor medical and diagnostic facilities and inadequate mosquito control. Therefore, studies like this will significantly contribute to the early diagnosis, management and can reduce patient's morbidity and mortality.

Keywords: Dengue Virus, Rapid diagnostic test, Thrombocytopenia

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Introduction

Dengue is a mosquito-borne viral disease caused by dengue virus belonging to the family flaviviridae and genus flavivirus. It is one of the most serious, rapidly spreading tropical diseases in the last five decades. The incidence has increased many folds with increasing geographic expansion from urban to rural population. Approximately 2.5 billion people live in dengue endemic countries with an estimated 50 million dengue infections at risk annually [1,2,3].

Dengue virus is small single-stranded RNA virus which is spherical in shape having 50 nm in diameter. It contains a host-derived membrane bilayer, a single copy of a positive-sense, single-stranded RNA genome and multiple copies of the three structural proteins and seven non-structural proteins [NS]. Dengue virus is divided in five types due to its characteristic antigenicity as DEN-1 to DEN-5. Out of these DEN-2 and DEN-3 known as "Asian" genotypes which are frequently associated with severe disease accompanying secondary dengue infections [4-8].

Now the diagnosis and confirmation of dengue viral infection can be done by viral culture, viral RNA detection by reverse transcriptase Polymerase Chain Reaction (RTPCR) and serological tests such as an immunoglobulin M (IgM) capture enzyme-linked immunosorbent assay (MAC-ELISA) [9].

In many dengue endemic remote areas with limited laboratory diagnostic services there rapid diagnostic tests are simple method which provides the immediate diagnosis and early management. The confirmation of these results can be done later as there is no antiviral treatment or vaccine available to prevent infection. But

careful fluid management and monitoring for complications is the only option available. As a result of early fluid management regimes and interventions, the case mortality rates can be decreased in many dengue endemic areas [10].

Materials and Methods

The present study was a retrospective study, conducted in the Pathology department of Dr. D. Y. Patil hospital at Pune from January 2016 to November 2016. Total 1236 serum samples were collected from clinically suspected cases of dengue out of which 500 samples were positive for either one or more of the dengue parameters. The samples were tested immediately in central laboratory for NS1 antigen, IgM and IgG antibodies by using the immunochromatography test kit- Dengue NS1 antigen and antibody using Combi Card supplied by well-known manufacturer.

The tests were performed in accordance with the manufacturer's instructions. Haematological parameters of all the dengue positive cases were recorded using haematology analyser. Statistical analysis was done by using Chi-square and Z-test wherever applicable.

Results

Table No.-1: Distribution of Dengue serological markers for Dengue infection [n=500]

Dengue specific parameters	Male	Female	Total positive serum samples	Percentage
NS1 only	179	117	296	59.2%
IgM only	16	5	21	4.2%
IgG only	87	64	151	30.2%
NS1 + IgM only	10	4	14	2.8%
NS1 + IgG only	7	4	11	2.2%
NS1 + IgM + IgG	1	1	2	0.4%
IgM + IgG	3	2	5	1%
Total	**303**	**197**	**500**	**100%**

The clinically suspected 1236 cases are tested at clinical laboratory in which 500 serum samples showed positivity for one or more markers like NS1, IgM or IgG out of which 303 are male and 197 were female. The majority of 296 (59.2%) cases were positive for only NS1 antigen, followed by IgG and IgM with 151 (30.2%) and 21 (4.2%) cases respectively [Table 1].

Table No.-2: Comparison of platelet count with various dengue parameters (n=500)

Dengue specific Parameters	Total positive serum samples	Platelet counts			
		Thrombocytopenia n= 345 [69%]			Normal n= 155[31%]
		<50,000/cumm)	50000 - 1 lakh/cumm	1 lakh - 1.5 lakh /cumm	>1.5 lakh/cumm
NS1 only	296	44	123	94	35
IgM only	21	3	5	8	5
IgG only	151	6	18	20	107
NS1 + IgM only	14	3	7	3	1
NS1 + IgG only	11	0	2	4	5
NS1 + IgM + IgG	2	0	2	0	0
IgM + IgG	5	0	2	1	2
Total	**500/**	**56**	**159**	**130**	**155**

All the suspected cases were screened for platelet count, WBC count and heamoglobin. Out of 500 seropositive cases 345 (69%) were showing thrombocytopenia (<1.5 lakh) while 155 (31%) cases are showing normal platelet count [Table 2].

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Table No.-3: Comparison of haemoglobin with various dengue parameters (n=500)

Dengue specific Parameters	Total positive serum samples	Haemoglobin		
		< 7.0gm%	<7.1 to9.9 gm%	>10.0gm%
NS1 only	296	1	6	289
IgM only	21	0	0	21
IgG only	151	1	4	146
NS1 + IgM only	14	0	0	14
NS1 + IgG only	11	0	0	11
NS1 + IgM + IgG	2	0	0	2
IgM + IgG	5	1	0	4
Total	**500**	**3**	**10**	**487**

Out of 500 cases study showed 487 (97.4%) cases with haemoglobin values more than 10gm%. Only 3 cases and 10 cases show haemoglobin range <7.0gm% and between 7.1-9.9 gm% respectively. [Table 3]

Table No.-4: Comparison of Total leukocyte count with various dengue parameters (n=500)

Dengue specific Parameters	Total positive serum samples	Total leukocyte count		
		4,000/cmm	4,000-11,000/cmm	>11,000/cmm
NS1 only	296	113	172	11
IgM only	21	6	14	1
IgG only	151	15	118	18
NS1 + IgM only	14	4	9	1
NS1 + IgG only	11	2	5	4
NS1 + IgM + IgG	2	0	2	0
IgM + IgG	5	1	4	0
Total	500	141	324	35

Study also showed 141 (28.2%) cases of leukopenia and 35 (7%) cases of leucocytosis. [Table no.4]

Table No.-5: Association of platelet counts with seropositivity in dengue infection

Platelet Count	Count Dengue Positive Cases	Dengue Negative Cases	Total
< 1,00,000/ml	215[43.0%]	187[25.4%]	402
> 1,00,000/ml	285[57.0%]	549[74.6%]	834
Total	**500[100%]**	**736[100%]**	**1236**
The P value is less than 0.0001. The association is considered to be statistically significant.			

Table No. 6 Association of platelet counts with NS1 positivity in dengue infection			
Platelet Count	< 1,00,000/ml	< 1,00,000/ml	Total
NS1 Positive	181	142	323
NS1 antigen negative	34	143	177
Total	**215**	**285**	**500**
The P value is less than 0.0001. The association is considered to be statistically significant.			

Discussion

In our study 323/500 (64.6%) cases are positive for NS1 antigen which are comparable to the study of Tathe S et al in which 56/93 (60%) were positive [11] Badave et al have shown 54//126 (42.9%) cases to be positive for NS1 [12]. Other studies by Kulkarni et al have shown NS1 positivity in 95/320 (30%) cases [13]. Datta and Shrivastava have shown that NS1 was positive in 140/600 (23.3%) and 15/91 (16%) cases respectively [14,15].

From 500 positive cases, 21 (4.2%) are positive for IgM only. This finding is comparable with studies of Badave et al and Sindhanai *et al* in which 6 (4.7%) and 9 (9.7%) were positive while as Tathe S et al reported it to be 16 (68.75%). Our study shows 151 (30.2%) cases to be positive for IgG only which is comparable to the study done by Badave et al and Sindhanai V *et al.* [11, 12, 16]

In Primary dengue case there will be a low titre and slow rising of antibodies. IgM antibody will appear first after 3-5 day followed by IgG antibody at the end of first week of illness. IgM levels peak about two weeks after the onset of symptoms and then decline generally to undetectable levels over 2–3 months. But anti-dengue serum IgG is increasing slowly after first week and there after it remains detectable after several months, and probably even for life. In contrast during secondary infection, rapid increase and high titre of antibodies are seen, i.e., high levels of IgG can be detected even during acute phase of secondary infection and IgM response is variable [1,17].

In our study, primary infection [positive for NS1 Ag, IgM, NS1 + IgM] was seen in 333 [66.7%] cases and secondary infection [positive for IgG, NS1 + IgG, IgM + IgG, NS1+ IgM+ IgG] was seen in 137 cases [33.3%]. (Table 1). Other studies by Golia et al. reported 57.4% primary dengue infections and 42.6% secondary dengue infections. Sindhanai V et al study showed primary infection in 59% cases and secondary dengue infections in 41% [16,18].

In Dengue fever the virus may interact and activate platelets leading to thrombocytopenia or may affect growth and differentiation of thrombopoiet in induced megakaryopoiesis inducing apoptotic cell changes in a subpopulation of early megakaryocytic progenitors. These events might contribute towards the origin of thrombocytopenia in dengue disease. In our study among 500 seropositive cases thrombocytopenia (<1 lakh) was evident in 215 (66.7%) which is comparable

to 68.8% in the study of Kulkarni et al and 81.1% in the study of Tathe et al Study. Out of 736 seronegative cases thrombocytopenia was seen in 187 (27.47%) and this association is considered to be statistically significant [Table no. 5]. This Reduction in platelet count observed in dengue negative cases may be due to other causes like viral infections other than dengue, vitamin deficiency, collagen vascular disorders, drug induced thrombocytopenia etc [19,20]. Out of 323 NS1 positive cases, thrombocytopenia was evident in 181(56.0%) and in exclusive antibody positive cases, thrombocytopenia was noted in 34 (19.2%) [Table No.6]. This association is considered to be statistically significant which is comparable to the study of Kulkarni et al, Sindhanai *et al* and Tathe et al Study.[11,13,16]

The cases with rapid decrease in platelet and white blood cell count with rising haematocrit levels are most likely to be due to dengue infection [1]. In our study out of 500 positive cases of dengue infection; leucopenia was evident in 141 (28.2%) cases and leucocytosis in 35 (7%) cases [Table No.6]. While the study done by Manimala S there were 57(57%) cases of leucopenia which is not comparable with our study. This disparity in results may be due to difference in sample size [21].

Conclusion and future

In this country the problem of dengue is complex due to the large population, poor medical and diagnostic facilities and inadequate mosquito control. This country needs a quick effective technique and reliable diagnosis in addressing this potentially fatal, epidemic prone infection and large number of virus laboratories to confirm it and alert the public to take action. Therefore, studies like this will significantly contribute to the clinical management and can reduce patient's morbidity and mortality. To conclude this study emphasises on the fact that dengue is a life-threatening condition and there is a need for early diagnosis and treatment in order to prevent its complications.

Author contribution

Author No. 1: Data compilation, processing and writing of manuscript.

Author No. 2: corresponding and statistical analysis

Author No. 3: final proof reading

Author No. 4: data collection, typing and formatting

Author No. 5: overall supervision and guidance

Author No. 6: typing and formatting

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