Journal of Cardiovascular Disease Research

ISSN: 0975-3583, 0976-2833 VOL 14, ISSUE 04, 2023

Original Research Article

DIFFERENTIATION OF PRIMARY AND SECONDARY DENGUE BY SIMULTANEOUS DETECTION OF NS1, IGM AND IGG BY ENZYME-LINKED IMMUNOSORBENT ASSAY: A CLINICO-SEROLOGICAL STUDY FROM SOUTH INDIA.

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Abstract

Introduction: Dengue is a highly infectious endemic disease of tropical countries caused by any of the five dengue virus serotypes: DENVs 1–4, transmitted within humans by the female Aedes mosquito. Dengue infection, ranges from mild asymptomatic dengue fever (DF) to fatal dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) which may turn fatal.

Aims and objectives: Secondary dengue infections are more severe than primary infection, hence, distinction between primary and secondary dengue is essential. Demonstration of anti DV IgG in patients' serum is a way to detect secondary dengue. The present study we explored the association of anti DV IgG positivity and dengue severity.

Materials and methods: This study was conducted in Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar, India. It was a prospective observational study done from July to December 2015 when the maximum numbers of dengue cases are recorded. Of the 2898 patients recruited

Results: Of the 2898 suspected dengue samples tested, 414 were positive for one or more of the three markers (NS1, IgM & IgG). 248 (59.9%) cases were anti DV IgM positive, 143 (34.5%) were Dengue NS-1 positives. Only 23 (5.5%) cases were having detectable anti dengue IgG in their serum (secondary dengue). Of the 414 cases, 391 (94.4%) had primary infection and 23 (5.5%) had secondary infection.

ISSN: 0975-3583, 0976-2833 VOL 14, ISSUE 04, 2023

Conclusion: In view of this, it is important to distinguish primary from secondary dengue early on in the course of illness. It helps in predicting prognosis and also in deciding; whether a patient needs admission and close monitoring or could be managed at home.
Keywords: Dengue virus, Primary dengue, Secondary dengue, Severe dengue, Thrombocytopenia, Dengue shock syndrome, ELISA

Introduction

Dengue viruses (DENVs) belongs to the family *Flaviviridae,* that are transmitted between humans globally by *Aedes* mosquitoes (*Aedes* [*Ae*.] *aegypti* and *Ae. albopictus*) that are found in tropical and subtropical environments.[1] Incidence of dengue has increased 30-fold in last five decades, currently, dengue is endemic to 128 countries. India is one of the countries maximally affected by it. A dramatic worldwide upsurge of the DENV has occurred due to rapid urbanization, increase in international travel, lack of effective mosquito control measures, and globalization.[2]

There are four dengue viral serotypes (DENV1–4), all the four serotypes differs genetically, but 65% of similarity can be observed among them.[3] Each serotype has several subtypes or genotypes. DENV-1 has three, DENV-2 has two, and DENV-3 and DENV-4 each have four. Each serotype has distinctive features and can present with severe manifestations depending on its interaction with the host response.[4] Infection with a particular kind of dengue serotype builds lifelong immunity to it, but the person is still vulnerable to be infected with the other types of dengue viruses.[5,6]

Although the pathogenesis of severe dengue remains incompletely understood, primary or first infection in nonimmune persons with one viral serotype usually causes DF. Subsequent dengue infection by a different serotype causes more severe illness, such as DHF/ DSS. The salient manifestations of DHF/DSS are sudden appearance of shock, capillary leakage, and hemorrhagic diathesis/ thrombocytopenia occurring at the time of defervescence of fever. Pathogenesis is due to more severe immunological response in the body.[5] Immune memory responses are neutralizing and largely protective to viruses of the same serotype upon re-exposure. They are also cross-reactive; however, prior infection provides protection against all 4 serotypes for only 2–3 months, after which protection is serotype-specific. When short term cross-protection wanes, patients with secondary DENV infections are at higher risk of severe disease.[6]

During secondary infection with a different serotype, or multiple infections with different serotypes, cross-reactive nonneutralizing antibodies bind to DENV and facilitate uptake via Fc receptors, resulting in enhanced viral replication. The resultant higher viral antigen load leads to an exaggerated activation of cross-reactive dengue specific T cells. Biological mediators released by the activated T cells as well as virus-infected cells along with complement activation by viral proteins and immune complexes are implicated in increasing vascular permeability and coagulopathy. This phenomenon is known as antibody dependent enhancement.[4]

Primary infection with any dengue virus serotype is characterized by an increase in virus specific IgM antibodies, 4-5 days after the appearance of clinical manifestations. While these are detectable in serum only for 30-90 days, the IgG antibodies persist for a long time, often for life. During a secondary infection however, the level of dengue virus specific IgM antibodies in serum is generally low and in some cases patients fail to show detectable amounts of these antibodies. But IgG levels rise rapidly to much higher levels

ISSN: 0975-3583, 0976-2833 VOL 14, ISSUE 04, 2023

than that observed during the primary dengue infection and often remain at these levels for at least 30-40 days. During primary infections, the specific IgG antibody response is initially of low avidity which gradually increases thereafter. On the contrary, secondary infections are characterized by an initial production of high-avidity antibodies. Due to this anomaly in the detection of dengue virus specific antibodies, the combined use of IgM and IgG has been proposed as an effective strategy for the serological differentiation of primary and secondary infections. Dengue virus specific IgG avidity ELISAs work on the basis of differences in IgM and IgG levels to distinguish between primary and secondary dengue fever.[7]

The early diagnosis of secondary dengue is important because it has been recognized as a significant risk factor for development of severe dengue.[5]

Materials and methods:

A prospective study was conducted at Chalmeda Anand Rao Institute of Medical Sciences Karimnagar, Telangana, from Jan 2020 to Dec 2021. Institutional Ethical Committee approval and informed consent form were obtained. A total of 2898 clinically suspected patients to have dengue fever as per WHO guidelines, were included in this study.

Inclusion Criteria:

Patients with oral temperature ≥ 38 °C, fever < 3 days associate with at least one of specific symptoms such as head ache, joint pain, back ache, abdominal pain, vomiting, fatigue, anorexia and diarrhoea) were included, as per WHO (2009).

Exclusion Criteria:

1. All the cases which were negative for serology, (or) positive for other causes of fever (Malaria, WIDAL, PUO), were excluded from the study.
2. Patients presenting with other co morbid infections along with dengue fever were not included into the study.
3. All the other causes of thrombocytopenia were also excluded from the study. All the other causes of thrombocytopenia were also excluded from the study. Seropositivity for dengue fever was determined using Microwell ELISA test for NS1, IgM and IgG.

Sample collection:

All the age groups suspected dengue fever were included in the study, sera collected within 3 days of onset of symptoms were referred to as acute-phase sera. A primary dengue case was defined as a laboratory-confirmed dengue infection with Dengue NS1 antigen ELISA(Tulip Diagnostics) and/or IgM capture ELISA positive and IgG capture ELISA negative. A secondary dengue case was defined as a laboratory-confirmed dengue infection with Dengue NS1 antigen ELISA and or IgM capture ELISA positive along with positive IgG capture ELISA. In addition to this, blood-total count (TC) and differential count (DC), erythrocyte sedimentation rate (ESR), haemoglobin (Hb), platelet count, renal and liver parameters, ultra sonogram, X-ray chest, and tests to rule out other causes of fever were done wherever necessary.

Results

Of the 2898 suspected dengue samples tested, 414 were positive for one or more of the three markers (NS1, IgM & IgG). 248 (59.9%) cases were anti DV IgM positive, 143 (34.5%) were Dengue NS-1 positives. Only 23 (5.5%) cases were having detectable anti dengue IgG in their serum (secondary dengue). Of the 52 paediatric (<14 years) cases, 16 (30.7%) were

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positive, of the 2846 adult (>14 years) cases, 398 (13.9%) were positive for one or more markers. A significant difference in incidence of dengue was observed between the adult and paediatric population ($P < 0.05$). Of this 16 paediatric cases, 2 (12.5%) were positive for IgG.

Of the 414 cases, 391 (94.4%) had primary infection and 23 (5.5%) had secondary infection. The difference in the incidence of primary and secondary dengue infection is statistically significant ($P < 0.001$). Among these 391 cases, NS1 alone was detected in 206 patients (49.7%) who came early within two days of illness and IgM in 127 (30.6%) patients who came on 3-5 days of illness.

Table-1: Distribution of primary & secondary serological markers among dengue positive cases

Primary & Secondary serological markers among dengue positive cases							
Serological marker	Days				**Total**	**Primary Infection**	**Secondary Infection**
	<2	3-5	6-7	>7			
NS1 Ag	78	91	37		206	206	
IgM		77	48	2	127	127	
IgG	3	6	2		11		11
NS1 and IgM		58			58	58	
NS1& IgG	1	3			4		4
IgM & IgG		1	2		3		3
NS1, IgM, IgG		1	4		5		5
	82	237	93	2	**414**	391	**23**

Table-2: Distribution of Cell count in primary & secondary cases(WBC and Platelets)

Cell count	**Primary Dengue 391(%)**	**Secondary Dengue 23(%)**
Thrombocytopaenia <1,00,000	239 (61.1%)	17 (73.9%)
Leukopenia (<4000/mm3)	22 (5.6%)	2 (8.6%)
Thrombocytopaenia & Leukopenia	57 (14.5%)	4 (17.39%)
Normal count	73 (18.3%)	0
Total Thrombocytopaenia	296 (75.7%)	21 (91.3%)
Total Leukopenia	79 (20.2%)	6 (26.0%)

Discussion

Laboratory confirmation of secondary dengue infection case is challenging. No standard method is available for differentiation of secondary dengue, WHO recommended hemagglutination inhibition (HI) test as the standard reference test by WHO to classify primary and secondary dengue virus infection. But this test requires paired serum samples, chemical pre-treatment long time, and high technical skills and exhibits high cross reactivity. Hence it is not amicable for every laboratory. Several studies have been performed IgG avidity test to discriminate among primary dengue and secondary dengue infection using the ratio of IgG and IgM at the different days of onset of symptom.[5,8,9]

ISSN: 0975-3583, 0976-2833 VOL 14, ISSUE 04, 2023

We used a well-established anti-dengue IgG capture ELISA. Primary infections are characterized by an increase in dengue specific IgM antibodies 4-5 days after the onset of febrile illness. Absence of dengue IgG in samples collected between days 0 and 8 makes it possible to define the case as primary infection and increased IgG antibodies after 7-10 days implies secondary infection. In secondary infections, IgG antibodies rise rapidly even during acute phase (before 7 days) of the disease. Hence concomitant detection of NS1, IgM, and IgG is essential to distinguish, secondary dengue from primary dengue depending upon the time of collection of specimens.

In the present study only 23 of 414 (5.5%) cases were secondary dengue. It is assumed that higher number of secondary dengue infections is seen in dengue endemic countries. In spite of the fact, that dengue is endemic in Telangana, India; most of the cases were of primary dengue, similar findings were reported in a study from Lucknow by Amita Jain et al.[10] We have a much higher percentage of males 246 (59.4%) compared to the females 168 (40.57%) and male to female ratio was 1.4: 1. In this study, 16 paediatric (<14 years) cases (30.7%) were positive for one or more dengue markers. This states that dengue is more common in children as observed in previous studies by, Poongodi S. Lakshmi, Gubler DJ., Gunasekaran P, Pancharoen C [11, 12, 13,14.]

NS1 is detectable from day 1 of fever both in primary and secondary infection. In the present study, of the 414 cases, NS1 alone was positive in 78 (18.8%) cases, NS1 Ag detection holds promising results in early diagnosis of dengue infection. This was observed as 16% by Shrivastava *et al*.,[14] Poongodi S.Lakshmi, 24%, Datta *et al*.,[15] 23% and Kulkarni *et al*.,[16] as 30%. This implies that all these cases might be misdiagnosed if NS1 marker is not included in ELISA test panel.

In secondary infection NS1 is detectable for shorter period as IgG antibody masks NS1 from detection or due to rapid clearance of NS1 in the form of the immune complexes. We observed positive triple markers (NS1, IgM, and IgG) positive in only 5 (1.2%) cases, cases and they were detected within 7 days representing secondary infection. This implies, they are either in the late stage of primary or secondary infection.

Primary infection is usually considered benign and severity in dengue is linked to the antibody dependent enhancement, initiated by secondary infection with a different serotype. In the present study 5 out of 12 patients needed hospitalization, but, all of them were discharged after successful management.

Fever and leukopenia, plus one or more of the list of symptoms in the World Health Organization (WHO) 2009 classification could be used for a clinical diagnosis of probable dengue, the current study establishes the association of dengue parameter positivity with thrombocytopenia. The comparison of platelet counts with different dengue specific parameter. Thrombocytopenia (platelet count <1lakh/ml, was observed in 296 (75.7%) among primary dengue and 21 (91.3%) in secondary dengue patients, similar findings were observed by Kulkarni et al.,[16] who observed this as 69% and 78% by Poongodi S. Lakshmi.[11] We observed Leukopenia (WBC count <4000/mm3 among 79 (20.2%) cases in primary dengue and 6 (26.0%)was observed in secondary dengue cases. Poongodi S. Lakshmi et al.,[11] observed 22% leukopenia in positive patients.

ISSN: 0975-3583, 0976-2833 VOL 14, ISSUE 04, 2023

The present study had certain limitations, as the study was conducted entirely from tertiary care hospital, larger proportion of severe cases were not included which might have influenced the proportion of secondary dengue cases.

Conclusion

Estimating the distribution of primary and secondary dengue is essential on several grounds, primarily, to identify geographical distribution for strengthening clinical management of dengue cases to reduce mortality associated with secondary dengue cases. We found the distribution of primary dengue cases were more prevalent than secondary dengue cases in a dengue endemic area of Telangana. We used ELISA based tests to differentiate secondary dengue, which is an affordable and more easily available in resource poor countries with dengue endemicity. Detection of Dengue NS1 antigen ELISA and or IgM capture ELISA was confirmed with anti-dengue IgG capture ELISA, concurrently with a single sample. Presence of anti Dengue IgG marker in patient's serum within 7 days of illness, is considered as an indicative of secondary dengue, suggests a high probability of more so progressing to severe disease, which significantly improves the diagnostic algorithm. Differentiation of primary and secondary dengue infection is essential to reduce morbidity and mortality due to DHF/DSS.

Acknowledgements

We thank all our laboratory staff Mr.M.Ravinder and Mr.P.Sagar for their unconditional support.

Conflict of Interest: Nill

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