

To differentiate primary and secondary dengue cases by IgG ELISA method in a tertiary care hospital at Rajasthan

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Abstract

Dengue remains a major arboviral infection in India with periodic outbreaks and considerable clinical burden. In 2017; NVBDCP reported 157220 positive dengue cases with 250 deaths in India. "In most cases, primary dengue infection runs a mild or self-limiting course. Severe manifestations such as dengue hemorrhagic fever and dengue shock syndrome are more commonly linked to immune responses triggered during secondary infections due to serotype cross-reactivity. Therefore, early recognition of secondary dengue infection at the initial phase of illness carries significant clinical value. "Thus, distinguishing primary from secondary dengue infections at an early stage is clinically important, as it carries significant prognostic value. The current study is a hospital based prospective analytical evaluation and was aimed to discriminate secondary from primary dengue virus infection in clinically suspected dengue cases presenting with fever and thrombocytopenia. Patients of all age groups attending SRG Hospital on outpatient and inpatient basis with clinically suspected dengue fever were included in the study. The samples were tested in the VRDL of the Microbiology Department for dengue NS1 antigen and IgM antibodies, positive for both were further subjected to IgG antibodies. IgM /IgG ratio was used to differentiate primary and secondary dengue infections. Dengue infection was categorized based on WHO guidelines. A total of 652 samples were tested from November 2021 to April 2024; out of 652 samples tested 215(32.97%) were positive for dengue NS1 and IgM. Of the 215 dengue NS1 and IgM positive cases, 75 (34.88%) were having secondary dengue infection, whereas 140 (65.11%) were having primary infection. Early detection of secondary infection helps the clinician in anticipating dengue related complications with appropriate therapeutic intervention, thereby reducing further complications and mortality.

Keywords: NS1 Antigen, IgM antibody, IgG antibody, thrombocytopenia

Introduction

Dengue fever is an acute mosquito-borne viral illness and the most common arboviral infection worldwide, with an estimated 390 million infections annually [1]. In India, the first epidemic resembling dengue was reported in Madras in 1780, while the first virologically confirmed outbreak occurred in Calcutta during 1963–1964 [2]. The disease is transmitted primarily by *Aedes aegypti*, a highly domestic mosquito, while *Aedes albopictus* plays a secondary role (3). Most Indian states are endemic, and recurrent outbreaks have been reported. According to the NVBDCP, 2, 33,251 cases with 303 deaths were recorded in 2022, [4, 5]. The dengue virus, belonging to the Flaviviridae family, comprises four antigenically distinct serotypes (DENV-1 to DENV-4). Infection may range from mild febrile illness to severe manifestations such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Secondary infections with heterologous serotypes are particularly associated with severe disease [6]. Thrombocytopenia (<100,000 cells/mm³) is an important marker of disease severity and requires careful monitoring, as it increases the risk of hemorrhagic complications [7]. Distinguishing between primary and secondary dengue infections is essential for clinical management, as secondary infections are more likely to progress to severe forms [6, 8]. The hem agglutination inhibition (HI) test is considered the reference standard but is limited by technical challenges, the need for paired sera, and poor early sensitivity [9]. In contrast, serological assays based on dengue-specific antibodies (IgM and IgG) are simpler and more practical. The IgM/IgG ratio serves as a reliable marker: a higher ratio indicates primary

infection, whereas a lower ratio indicates secondary infection [10]. This parameter is especially valuable in resource-limited settings where sophisticated diagnostic tools may not be available. In this context, the present study was designed to differentiate primary and secondary dengue infections among clinically suspected patients presenting with fever and thrombocytopenia

Methods

This study was carried out in the Viral Research and Diagnostic Laboratory (VRDL), Department of Microbiology, Jhalawar Medical College and SRG Hospital, Jhalawar. Ethical approval for this study had been obtained and as dengue testing forms part of the routine diagnostic services, no separate clearance was required for this study.

Study design and Participants

A consecutive sampling approach was followed. From November 2021 to April 2024, a total of 652 patients clinically suspected of dengue fever were included. Demographic, clinical, hematological, and serological information was recorded for all cases. Individuals of any age presenting with fever were enrolled for this study. The duration of illness was determined from the onset of clinical symptoms such as fever, myalgia, vomiting, and retro-orbital pain.

Sample Collection and Processing

Venous blood was collected in clot activator vacutainers and transported to the Virology Research and Diagnostic Laboratory (VRDL) within two hours. Serum was separated

and tested within 24 hours. All samples were screened for dengue NS1 antigen and IgM antibodies; those positive for either were further tested for IgG, and the IgM/IgG ratio was calculated to differentiate primary from secondary infection. “The total number of positive cases was derived by adding the NS1-positive and IgM-positive samples and subtracting the samples that tested positive for both NS1 and IgM, as all specimens were systematically tested for both markers.”

Diagnostic assays

NS1 antigen was detected using the Dengue NS1 Ag Microlisa kit (J. Mitra & Co. Pvt. Ltd.). IgM antibodies were identified with the NIV MAC-ELISA (National Institute of Virology, Pune), and IgG antibodies with the Panbio Dengue IgG Indirect ELISA (Abbott Diagnostics). All tests were performed according to manufacturer protocols.

Case definitions

- **Thrombocytopenia:** Platelet count <100,000 cells/mm³.
- **Primary and secondary dengue infection:** Differentiation was based on WHO guidelines using NS1, IgM, IgG, and the IgM/IgG ratio of samples collected during the acute phase
- **Primary infection:** NS1 and IgM positive with IgG negative, or IgM/IgG ratio >1.2.
- **Secondary infection:** NS1, IgM, and IgG positive with IgM/IgG ratio <1.2.
- **Past infection:** IgG positive with NS1 and IgM negative.

Table 1: WHO criteria for differentiating primary and secondary infection

Result of NS1 Antigen	Result of IgM Antibody	Result of IgG Antibody	Ratio of IgM/IgG	Classification of dengue
Positive	Positive	Negative	NA	Primary infection
Negative	Negative	Positive	NA	Previous infection
Positive	Positive	Positive	Greater than 1.2	Primary infection
Positive	Positive	Positive	Less than 1.2	Secondary infection

Table 2: Year-wise distribution of positive cases

Year	Samples Tested	Total positives	Only IgM positives	NS1 and IgM positives	Dengue secondary infection	Dengue Primary Infection
2021	84	55	24	14	20	35
2022	184	19	17	02	09	10
2023	292	106	28	17	31	75
2024	92	35	05	04	15	20
Total	652	215	74	37	75	140

“In the above table : The total number of positive cases was calculated as the sum of NS1-positive and IgM-positive samples, after subtracting those that were concurrently

positive for both NS1 and IgM, since all samples were uniformly tested for both markers.”

Table 3: Age-wise distribution of cases in primary and secondary infection

Age wise distribution years	Total cases	Total Positive	Primary Infection	Secondary Infection
< 15 years	44	17	11(64.70%)	06(35.29%)
15-30 years	257	98	66 (67.34%)	32 (32.65%)
31-45 years	166	46	30 (65.21%)	16 (34.78%)
45-60 years	90	22	13 (59.09%)	09 (40.90%)
>60 years	95	32	20 (62.5%)	12 (37.5%)
Total	652	215	140 (65.11%)	75 (34.88%)

Table 4: Demographic and Laboratory data with respect to primary and secondary infection

Demographic and laboratory data	Total	Primary	Secondary
Male	114	69	45
Female	101	71	30
Mean Age	34.4	33.9	35.4
Mean IgM OD	0.483	0.275	0.533
Mean IgG OD	1.714	0.238	2.071
Mean Platelet count	89646.7	116454.5	84385.5

Table 5: Platelet count in primary and secondary infection

Thrombocytopenia	Total	Primary Infection	Secondary Infection
>50000	15 (7.0%)	10 (4.7%)	05 (2.3%)
51000-100000	147 (68.4%)	96 (44.7%)	51 (23.7%)
101000-150000	53 (24.6%)	34 (15.8%)	19 (8.8%)
Total	215 (100%)	140 (65.1%)	75 (34.9%)

Results

The present study analyzed 652 suspected dengue cases tested during the study period. Out of these, 215 (32.9%) were confirmed positive for dengue either by NS1 antigen and/or IgM antibody ELISA. Of the positives, 74 (34.4%) were IgM-only positive, while 37 (17.2%) were positive for both NS1 and IgM. Based on IgG testing and WHO criteria, 140 (65.1%) were classified as primary infection and 75 (34.9%) as secondary infection (Tables 1 & 2).

All age groups were affected, with the 15–30 years group showing the highest positivity (98/215; 45.6%). Primary infection predominated across all age categories, though the highest proportion of secondary infection (6/17; 35.3%) was observed among those aged <15 years (Table 3).

Males accounted for 114 (53.0%) cases and females for 101 (47.0%), showing almost equal distribution. The mean age was 33.9 years in primary and 35.4 years in secondary infections. Mean IgM OD was higher in secondary (0.533) compared to primary infections (0.275), while mean IgG OD was markedly elevated in secondary (2.071) compared to primary (0.238) (Table 4).

Thrombocytopenia was common in both groups, with most cases having platelet counts between 50,000–100,000/mm³ (147/215; 68.4%). Severe thrombocytopenia (<50,000/mm³) was more frequent in secondary infections (5/75; 6.7%) than in primary infections (10/140; 4.7%) (Table 5)

Discussion

In the present study, 215 (32.9%) of 652 suspected cases tested positive for dengue infection. Of these, 140 (65.1%) were classified as primary infection and 75 (34.9%) as secondary infection based on IgM/IgG ratios. Both males and females were almost equally affected (M: F ratio 1.06:1), which is consistent with reports from South India where similar trends were observed [10]. The nearly equal distribution can be attributed to the breeding habits of *Aedes aegypti*, which thrives both indoors and outdoors [3].

The 15–30 years age group accounted for most cases, with a mean age of 34.4 years. This trend aligns with earlier reports from endemic regions of India and Southeast Asia (1, 11). The persistence of high transmission among young adults supports the endemicity of dengue in southern India, consistent with national data showing a steady increase since 2014 [5].

Thrombocytopenia was a common feature among dengue-positive cases in our study. Secondary infections were more frequently associated with platelet counts below 100,000/mm³, echoing previous studies that have highlighted the relationship between secondary infection and more severe hematological manifestations [8, 12].

Severe thrombocytopenia (<50,000/mm³) was observed in both groups but was slightly more frequent in secondary infections, reinforcing the clinical relevance of distinguishing between primary and secondary dengue. The immunopathogenesis of severe thrombocytopenia in secondary infections has been explained by antibody-dependent enhancement, which facilitates increased viral replication and heightened immune activation [6, 7]. The resulting cytokine surge contributes to vascular leakage and hematological abnormalities. In addition, platelet destruction has been linked to immune-mediated mechanisms, including activation by nonstructural protein 1 and antiplatelet antibodies [14].

The IgM/IgG ratio remains a practical approach for differentiating primary and secondary infections. Studies from India have validated a cut-off ratio of approximately 1.5, demonstrating good sensitivity and specificity for classification [9, 13]. Our findings confirm its clinical usefulness, particularly when combined with NS1 and IgM detection, as recommended in current diagnostic guidelines [4].

Conclusion

Our study highlights the importance of secondary dengue infection (80.5%) in a tertiary care setting, with a strong association with thrombocytopenia. The IgM/IgG ratio, in combination with NS1 and IgM ELISA, provides a reliable and cost-effective means of differentiating primary and secondary infections. This is crucial for timely diagnosis, risk stratification, and management in endemic regions. Incorporating comprehensive dengue testing (NS1, IgM, IgG, and IgM/IgG ratio) into diagnostic protocols, even in resource-limited settings, will aid in reducing morbidity and improving outcomes.

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