Original Article

Assessment of Molecular Prevalence of Dengue Serotypes Among the Viremia Cases of YSR Kadapa District of Andhra Pradesh, India by TaqMan Assay

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Abstract

Background: Dengue is a major public health concern in India, with dynamic shifts in circulating serotypes influencing outbreak patterns and disease severity. Molecular surveillance plays a crucial role in identifying dominant serotypes during viremic phases of infection. The objective is to determine the molecular prevalence and distribution of dengue virus serotypes among NS1-positive viremic cases in YSR Kadapa district and to assess concordance between NS1 ELISA and RT-PCR. **Material and Methods:** A cross-sectional study was conducted at a tertiary care teaching center. Serum samples from NS1 antigen-positive patients were analyzed by serotype-specific real-time RT-PCR with TaqMan probes. Demographic distribution and serotype prevalence were analyzed. Chi-square test was used to assess associations between age groups and serotype distribution. **Results:** Among RT-PCR–confirmed cases, DENV-3 was the predominant serotype (75%), followed by DENV-2 and DENV-4. Co-circulation of multiple serotypes was observed, indicating a hyperendemic transmission pattern. The highest proportion of cases occurred in the 0–10-year age group. No statistically significant association was found between age group and serotype distribution ($p > 0.05$). A discordance rate of 36% was observed between NS1 ELISA and RT-PCR results. **Conclusion:** The predominance of DENV-3 and co-circulation of other serotypes highlight ongoing serotype dynamics in YSR Kadapa district. Periodic molecular surveillance at designated laboratories may enhance outbreak detection and epidemiological monitoring in endemic regions.

Keywords: Dengue virus; Serotype distribution; Real-time RT-PCR; NS1 antigen; Molecular surveillance.

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Introduction

Dengue virus (DENV) infection is one of the most rapidly spreading mosquito-borne viral diseases worldwide and represents a major public health challenge in tropical and subtropical regions.[1] It is estimated that 100–400 million dengue infections occur annually, with a substantial proportion resulting in severe clinical manifestations such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).[2] Dengue virus belongs to the genus Flavivirus within the family Flaviviridae and is transmitted primarily by Aedesaegypti mosquitoes.[3] The increasing frequency of outbreaks, expanding geographic distribution, and rising disease severity have made dengue a priority disease for surveillance and control programs globally.[1,4]

Dengue virus exists as four antigenically distinct serotypes, namely DENV-1, DENV-2, DENV-3, and DENV-4, all of which can cause clinical disease.[5] Infection with one serotype confers lifelong immunity to that serotype but only transient protection against others, making individuals susceptible to secondary infections.[6] Secondary dengue infections are associated with antibody-dependent enhancement, which significantly increases the risk of severe disease outcomes.[7] Studies from India have demonstrated frequent shifts in circulating serotypes, with regional variations influencing outbreak intensity and disease severity.[8,9] Therefore, understanding serotype distribution is critical for predicting epidemic potential and guiding public health interventions.[10]

India is hyperendemic for dengue, with all four serotypes circulating in different parts of the country.[8] Several molecular epidemiological studies from Central and South India have reported the emergence and predominance of specific serotypes, particularly DENV-2 and DENV-3, during recent outbreaks.[9,11] Andhra Pradesh has experienced recurrent dengue outbreaks; however, data on molecular serotype distribution at the district level remain limited.[12] In the study region, despite annual increases in dengue cases, there is a lack of systematic

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molecular surveillance data, creating a gap in understanding local transmission dynamics and serotype circulation patterns.[12,13]

Early and accurate identification of dengue virus serotypes is vital for outbreak investigation, disease severity assessment, and the implementation of targeted vector control measures.[5] While serological assays, such as NS1 antigen detection, are useful for early diagnosis, molecular techniques, such as real-time reverse transcription polymerase chain reaction (RT-PCR), provide superior sensitivity and enable precise serotype identification during the viremic phase.[14] The TaqMan real-time RT-PCR assay has been widely adopted for dengue surveillance due to its high specificity, rapid turnaround time, and ability to detect low viral loads.[15] In this context, the present study was undertaken to assess the molecular prevalence and serotype distribution of dengue virus among viremic cases in the study region using a TaqMan-based RT-PCR assay to generate region-specific epidemiological data and support local dengue control strategies.[9,12]

Materials and Methods

Study Design and Setting: This cross-sectional, laboratory-based study was conducted at the Department of Microbiology in collaboration with the Department of General Medicine at Rajiv Gandhi Institute of Medical Sciences (RIMS), Kadapa, Andhra Pradesh, India. The study included clinically suspected dengue cases presenting with acute febrile illness and features suggestive of viremia. The study was carried out during the defined study period after obtaining institutional approval.

Study Population and Sample Size: A total of 100 consecutive patients who tested positive for dengue NS1 antigen by rapid immunochromatographic test (RDT) were included in the study. Patients of all ages and both sexes presenting with fever of less than 7 days' duration and clinical suspicion of dengue infection were eligible for inclusion.

Inclusion criteria were

(i) patients presenting with acute febrile illness clinically suspected of dengue; (ii) NS1 antigen positivity by rapid diagnostic test; (iii) availability of an adequate serum sample for molecular testing; and (iv) provision of written informed consent (or parental consent for minors).

Exclusion criteria included:

(i) NS1-negative cases; (ii) patients with confirmed alternative diagnoses such as malaria, typhoid, leptospirosis, or bacterial sepsis; (iii) patients with respiratory viral infections; (iv) inadequate, hemolyzed, or improperly stored samples; and (v) refusal to provide consent.

Ethical Approval: Ethical clearance was obtained from the Institutional Ethics Committee (IEC). Written informed consent was obtained from adult participants, and assent with parental consent was obtained for pediatric cases. All procedures were performed in accordance with institutional ethical standards.

Sample Collection and Processing: Approximately 3–5 mL of venous blood was collected under aseptic conditions. Serum was separated by centrifugation at 3,000 rpm for 10 minutes and stored at −80°C until further analysis. All sample handling was performed under Biosafety Level 2 (BSL-2) conditions, in accordance with WHO guidelines.

Serological Testing: All RDT-positive samples were further tested using a commercially available NS1 antigen ELISA kit (J. Mitra& Co. Pvt. Ltd., India) according to the manufacturer's instructions. Optical density was measured at 450 nm using a microplate reader. Samples with absorbance values above the calculated cut-off were considered positive for dengue NS1 antigen and indicative of active infection.

Viral RNA Extraction: Viral RNA was extracted from NS1 ELISA-positive serum samples using the QIAamp Viral RNA Mini Kit (Qiagen, Germany) following the spin-column method as per the manufacturer's protocol. RNA was eluted in RNase-free buffer and stored at −80°C until amplification.

Real-Time RT-PCR and Serotyping: Detection and serotyping of dengue virus RNA were performed using one-step real-time reverse transcription polymerase chain reaction (RT-PCR) with serotype-specific primers and TaqMan probes, according to the CDC DENV-1–4 protocol. Amplification was carried out using the SuperScript™ III Platinum® One-Step Quantitative RT-PCR Master Mix (Thermo Fisher Scientific, USA) on a Bio-Rad CFX96 Touch Real-Time PCR system.

Each reaction was performed in a total volume of 25 µL containing 5 µL of extracted RNA, master mix, primers, probes, enzyme mix, and nuclease-free water. Reverse transcription was performed at 55°C for 30 minutes, followed by initial denaturation at 95°C for 3 minutes and 40 amplification cycles (95°C for 15 seconds and 60°C for 1 minute). A cycle threshold (Ct) value <35 was considered positive.

Quality Control: Positive and negative controls were included in each RT-PCR run. RNA extraction, reagent preparation, and amplification were performed in separate designated areas to prevent cross-contamination. All assays were performed in triplicate to ensure reproducibility.

Statistical Analysis: Data were entered into Microsoft Excel and analyzed using SPSS version 26.0 (IBM Corp., USA). Descriptive statistics were used to summarize demographic characteristics and serotype distribution. Associations between categorical variables (age group, gender, and geographic distribution) and dengue serotypes were assessed using the Chi-square (χ^2) test or Fisher's exact test where appropriate. A p-value <0.05 was considered statistically significant.

Results

A total of 100 clinically suspected dengue cases tested positive for NS1 antigen by rapid diagnostic test and were included in the study. Of these, 64 samples (64%) were confirmed positive by NS1 ELISA and subsequently subjected to real-time RT-PCR for molecular confirmation and serotype identification.

RT-PCR detected dengue viral RNA in 64 cases (64%), whereas 36 samples (36%) failed to amplify despite NS1 positivity. The overall molecular confirmation rate among NS1-positive cases was 64%.

Table 1: Laboratory Confirmation of Dengue Cases (n = 100)

Test Modality	Positive n (%)	Negative n (%)	χ^2 value	p-value
NS1 ELISA	64 (64%)	36 (36%)	7.84	0.005*
RT-PCR	64 (64%)	36 (36%)	7.84	0.005*

Chi-square test comparing positive vs negative proportions; statistically significant

Serotype analysis of the 64 RT-PCR–confirmed cases demonstrated a clear predominance of DENV-3.

Table 2: Distribution of Dengue Virus Serotypes Among RT-PCR Positive Cases (n = 64)

Serotype	Number (n)	Percentage (%)	χ^2 value	p-value
DENV-1	1	1.6%	82.56	<0.001*
DENV-2	7	10.9%		
DENV-3	48	75.0%		
DENV-4	8	12.5%		
Total	64	100%		

Chi-square test comparing distribution among serotypes; statistically significant predominance of DENV-3.

DENV-3 accounted for 75% of cases (48/64), followed by DENV-4 (12.5%), DENV-2 (10.9%), and DENV-1 (1.6%). The difference in serotype distribution was statistically significant ($p < 0.001$), indicating dominance of DENV-3 during the study period.

Table 3: Age-wise Distribution of Dengue Serotypes (n = 64)

AgeGroup (Years)	TotalCases (n)	DENV-1	DENV-2	DENV-3	DENV-4	χ^2 value	p-value
0–10	18	0	2	14	2	4.12	0.845
11–20	12	0	1	9	2		
21–30	11	1	1	8	1		
31–40	9	0	1	7	1		
41–50	8	0	1	6	1		
≥51	6	0	1	4	1		

Chi-square test for association between age group and serotype distribution; not statistically significant.

There was no statistically significant association between age group and serotype distribution ($p > 0.05$).

Table 4: Concordance Between NS1 ELISA and RT-PCR (n = 100)

NS1 ELISA	RT-PCR Positive	RT-PCR Negative	Total	χ^2 value	p-value
Positive	64	36	100	7.84	0.005*

Chi-square test assessing concordance between ELISA and RT-PCR; statistically significant discordance observed.

Using RT-PCR as the reference standard, NS1 ELISA demonstrated 100% sensitivity and a positive predictive value of 64%. However, specificity and negative predictive value could not be reliably calculated, as only NS1-positive cases were included in the study.

Discussion

The present study demonstrated a clear predominance of DENV-3 (75%) among RT-PCR–confirmed cases in the study region, indicating active circulation of this serotype during the study period.[16] Similar dominance of DENV-3 has been reported from several regions of Central and South India, suggesting cyclical shifts in circulating serotypes in endemic settings.[8,11,16] Such serotype replacement patterns are epidemiologically significant, as outbreaks often intensify when population-level immunity to the emerging dominant serotype is low.[20]

The concurrent detection of DENV-2 and DENV-4, though at lower frequencies, reflects the hyperendemic nature of dengue transmission in India, where multiple serotypes co-circulate simultaneously.[8,17] Co-circulation increases the likelihood of secondary heterotypic infections, which are strongly associated with severe clinical manifestations due to antibody-dependent enhancement mechanisms.[6,7,17] Although clinical severity was not systematically analyzed in this study, the presence of multiple serotypes highlights the potential risk for more severe outbreaks in subsequent transmission cycles.

Age-wise distribution revealed a higher burden of infection in children aged 0–10 years, with DENV-3 predominating across all age groups.[18] Pediatric predominance has been documented in other Indian studies and may be attributable to early exposure, lower pre-existing immunity, and environmental factors influencing vector contact.[13,18] The absence of a statistically significant association between age and serotype distribution suggests uniform transmission across demographic groups, consistent with endemic transmission dynamics.[10]

Methodologically, TaqMan-based real-time RT-PCR enabled precise serotype identification during the viremic phase of infection.[15] Molecular detection during early illness provides greater specificity for active infection compared to serological assays alone and allows timely characterization of circulating strains.[5,14] By focusing on NS1-positive cases, the study targeted patients likely to be within the diagnostic window of viremia, thereby enhancing serotyping accuracy.

A 36% discordance between NS1 ELISA and RT-PCR was observed, which may reflect differences in the temporal kinetics of NS1 antigenemia and viral RNA detectability.[14,19] NS1 antigen can persist beyond peak viremia, whereas RNA levels may decline depending on the stage of illness at

presentation.[19]These findings underscore the importance of interpreting diagnostic results within the context of illness duration and highlight the complementary roles of serological and molecular methods in dengue surveillance.

Strengthening molecular surveillance through periodic serotype monitoring at designated public health laboratories may improve early outbreak detection and epidemiological tracking in high-burden districts such as YSR Kadapa.[12,21] Continuous monitoring of serotype dynamics is essential for anticipating epidemiological shifts, guiding vector control strategies, and informing public health preparedness measures in endemic regions.[10,20]

Strengths

This study provides region-specific molecular epidemiological data on circulating dengue serotypes from YSR Kadapa district, where limited published data are available. The use of serotype-specific TaqMan real-time RT-PCR ensured accurate detection and differentiation of dengue serotypes during the viremic phase. Inclusion of multiple age groups enabled demographic analysis of serotype distribution. The study also highlights diagnostic discordance between NS1 ELISA and RT-PCR, offering practical insights for optimizing dengue surveillance strategies in endemic settings.

Limitations

The study included only NS1-positive cases, potentially excluding patients presenting later in the illness, thereby limiting a comprehensive assessment of diagnostic performance. The sample size was modest and derived from a single tertiary care center, potentially affecting generalizability. Detailed clinical severity correlation and viral load quantification were not performed. Additionally, phylogenetic or whole-genome sequencing was not undertaken to assess genetic diversity among circulating strains.

Conclusion

The present study demonstrates the predominance of DENV-3 among viremic dengue cases in YSR Kadapa district, with evidence of co-circulation of DENV-2 and DENV-4, indicating a hyperendemic transmission pattern. The findings highlight ongoing serotype shifts and underscore the epidemiological importance of periodic molecular surveillance to monitor circulating strains. The discordance between NS1 ELISA and RT-PCR underscores the need to interpret diagnostic results appropriately based on the stage of illness. Targeted serotype monitoring at referral laboratories may strengthen outbreak preparedness and guide public health interventions in endemic regions.

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Conflicts of interest

There are no conflicts of interest.

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