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Molecular Study on Dengue Viruses of Patients in A Tertiary Care Hospital in Western Odisha

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ABSTRACT

Background: As a viral disease caused by the Flavivirus transmitted by mosquitoes, Dengue is a common illness in tropical and subtropical countries with clinical manifestations from mild fever to complicated forms like Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS). Early diagnosis and specificity are important because there is no specific antiviral therapy or licensed vaccine available.

Aim: To assess the usefulness of molecular diagnostics in dengue virus serotyping and correlate results with clinical and demographic profiles.

Materials and Methods: 384 clinically suspected dengue patients according to WHO criteria were included. Serum was screened for antigen and antibodies by rapid tests and ELISA. Samples that were NS1 positive were also subjected to RT-PCR for detection of viral RNA and serotyping.

Results: 70 ELISA positives were seen out of 384 samples. RT-PCR verification of DENV-1 and DENV-2 serotypes was seen, with the predominance of DENV-2. Infection was mostly seen in males, and among the male population, 15–30 years was most affected. The post-monsoon season saw the maximum cases, specifically in the month of October. The most frequent symptoms that were seen were fever, headache, myalgia, arthralgia, and manifestations of bleeding.

Conclusion: ELISA is still a useful screening tool, and RT-PCR is useful for precise serotyping for effective surveillance. DENV-2 predominance with more severe clinical manifestations highlights the importance of ongoing vigilance and early detection, especially during peak risk periods.

Keywords: *Dengue virus, Serotyping, ELISA, RT-PCR, DENV-2, Molecular diagnostics*

1. INTRODUCTION

Dengue is the most significant vector-borne viral disease, primarily spread by the Aedes aegypti mosquito, and a public health issue of immense concern in the tropics and subtropics. It accounts for approximately 17–20% of infectious diseases worldwide. India, particularly in the past few years, has witnessed large-scale epidemics of dengue, often in co-epidemics with other arboviral diseases like chikungunya [1,2].

DENV is a member of the Flaviviridae family and is an RNA virus with five known serotypes (DENV-1 to DENV-5). The disease ranges from mild dengue fever (DF) to severe manifestations such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Secondary infection with a new serotype can lead to severe disease due to antibody-dependent enhancement (ADE) [3,4].

Laboratory investigations confirm clinical dengue diagnosis. Diagnosis is facilitated at an early stage by detection of non-structural protein 1 (NS1) antigen and IgM and IgG antibodies that differentiate primary and secondary infections [5,6]. Molecular diagnostic assays like multiplex real-time PCR play a vital role in confirmation of diagnosis and identification of circulating serotypes [7].

The current study was conducted in a tertiary health care facility in Western Odisha to evaluate the effectiveness of NS1 antigen test for early diagnosis and identification of circulating dengue virus serotypes by multiplex RT-PCR and correlation with severity of the disease [8].

2. METHODOLOGY

Research Methodology and Context

This study was a prospective observational study conducted at the Veer Surendra Sai Institute of Medical Sciences and Research (VIMSAR), Burla, between February 2021 and December 2022. The study included patients presenting with clinical symptoms that suspected dengue infection in the outpatient departments of both Pediatrics and Medicine. The study protocol was reviewed and cleared by the Institutional Ethics Committee, and informed consent was taken from all the participants before sample collection.

Study Demographics and Sample Magnitude

The study comprised rural and urban Western Odisha patients with signs and symptoms suggestive of dengue infection. Inclusion criteria were per World Health Organization guidelines and comprised all ages and both sexes of patients with clinical features of fever, myalgia, arthralgia, or bleeding manifestations. Patients with other confirmed diagnosis (e.g., malaria, dengue, chikungunya), patients undergoing treatment for platelet disorders or other viral infections, and participants not willing to participate or whose samples got contaminated were excluded from the study. According to prior research, which had estimated the prevalence rate of dengue at 32.6%, the sample size was calculated at 340 using the appropriate statistical formula.

Sample Collection and Serum Processing

Venous blood samples of 4–5 mL were collected under aseptic precautions in red-capped plain vacutainers. The samples were allowed to clot at room temperature and subsequently centrifuged at 3000 rpm for 10 minutes. The obtained serum was carefully separated and stored in duplicate in cryochill vials. One aliquot was immediately used for rapid testing, and the other was stored at –80°C for subsequent ELISA and molecular tests.

Serological Diagnosis

For presumptive diagnosis, serum samples were taken for rapid diagnostic testing using the Dengue Day 1 Test Kit (J. Mitra & Co., New Delhi, India), which detects NS1 antigen and IgM and IgG antibodies. The test was performed according to the manufacturer's instructions and results were interpreted as positive or negative based on the visibility of pink bands in specific areas of the test card.

Further confirmation of dengue infection was done through ELISA methods. NS1 antigen was identified with the Merilisa NS1 ELISA kit and IgM antibodies with the NIV Dengue IgM Capture ELISA kit. NS1 ELISA involved incubation of patient and control sera with microtiter plates coated with anti-Dengue NS1 antibody. Following a series of incubation, wash, and colorimetric development cycles, absorbance was read at 450 nm using an ELISA reader. Reactive samples were those with absorbance values above the preset cutoff. The IgM ELISA had the same protocol with antigen capture, antibody binding, and enzyme-conjugate reaction. The test was interpreted from the relative absorbance values between the sample and negative control with preset thresholds of positivity or equivocal results.

Molecular Detection by RT-PCR

The NS1 antigen positive samples were forwarded to ICMR-RMRC at Bhubaneswar for molecular confirmation and serotyping by multiplex real-time reverse transcription polymerase chain reaction (RT-PCR). The primary aim was to detect the RNA of the dengue virus as well as determine the specific serotypes prevailing.

Total RNA was isolated from serum using the QIAamp Viral RNA Mini Kit (QIAGEN, Valencia, CA) as per the manufacturer's instructions. The procedure involved cell lysis, binding of viral RNA to a silica membrane, multiple washes to remove contaminants, and the final elution of the purified RNA into nuclease-free water. The isolated RNA was then refrigerated at –70°C for analysis.

The RT-PCR was optimized to identify conserved serotype-specific dengue virus genome regions. Full-genome alignments obtained from the NCBI database were used as the basis for designing primers and DENV-1 to DENV-4 fluorogenic probes. BLAST analyses were used to verify that the sequences were specific. Amplification was performed in a 25 µL reaction mixture with the TaqMan Fast Virus 1-step master mix (Applied Biosystems®) supplemented with 5 µL of template RNA and serotype-specific primers and probes. The RT-PCR reaction cycle involved reverse transcription at 50°C for 30 minutes, inactivation of the enzyme at 95°C for 2 minutes, and followed by 45 cycles of amplification, with denaturation at 95°C and annealing at 60°C. Fluorescence detection was performed on an ABI 7500 real-time PCR machine. Each dengue serotype was identified by a unique fluorophore-labeled probe, allowing one-reaction simultaneous detection. Negative controls, such as RNA from other flaviviruses and no-template controls, were employed to determine specificity. A sample was considered positive when amplification was seen within 40 cycles. By employing a multi-pronged strategy involving the use of rapid

diagnostics, ELISA, and RT-PCR, the research sought to facilitate the early diagnosis of dengue infections and establish the prevalence of viral serotypes in the region.

Ethical Considerations

The research work "Molecular Study on Dengue Viruses of Patients in a Tertiary Care Hospital in Western Odisha" had the approval of the Institutional Research and Ethics Committee (IREC) of VIMSAR, Burla (Reg. No. EC/NEW/INST/2021/2010), and was finally approved on 17.05.2022. The research was supervised by Dr. Sudipta Kumar Ram and got clearance for one year as per full review. Informed consent was received from all the participants and ethical guidelines were maintained during the research according to the Declaration of Helsinki and national guidelines for biomedical research.

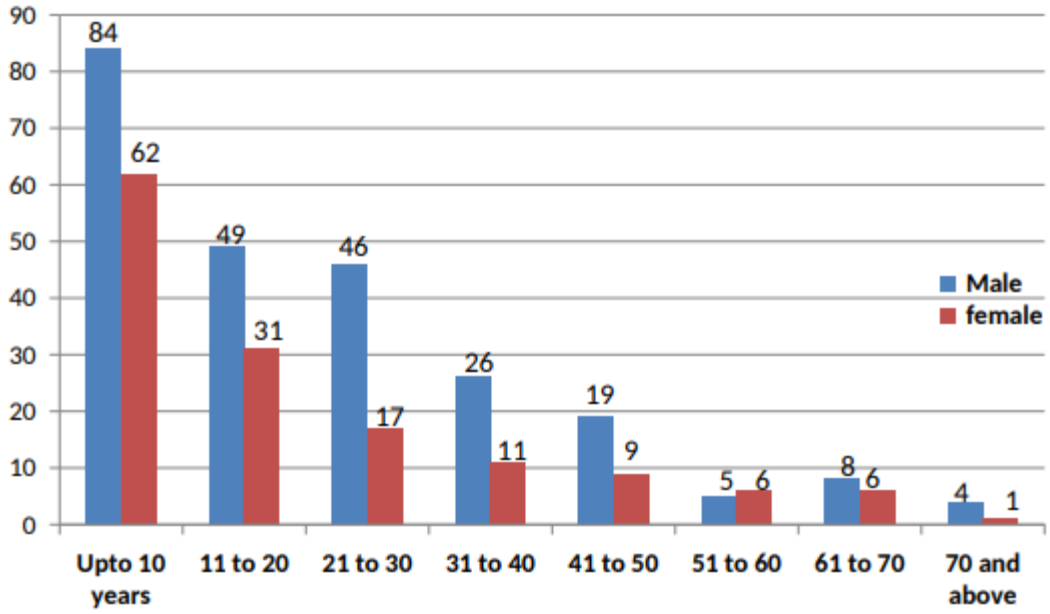
3. RESULT

Overall Study Population

A total of 384 suspected dengue cases (based on WHO criteria: fever plus ≥1 additional symptom) were analyzed from 2021‑2022. Males predominated (242; 63.0%), with a male-to-female ratio of 1.7:1. The highest proportion of cases occurred in children ≤10 years (38.0%), followed by 11‑20 years (21.0%).

Table 1: Demographic Distribution of Suspected Dengue Cases

Age Group (Years)	Male, n (%)	Female, n (%)	Total, n (%)
≤10	84 (57.0)	62 (43.0)	146 (38.0)
11–20	49 (62.0)	31 (38.0)	80 (21.0)
21–30	46 (73.0)	17 (27.0)	63 (16.0)
31–40	26 (70.0)	11 (30.0)	37 (9.0)
41–50	19 (67.0)	9 (33.0)	28 (7.0)
51–60	5 (45.0)	6 (55.0)	11 (3.0)
61–70	8 (57.0)	6 (43.0)	14 (3.6)
≥70	4 (80.0)	1 (20.0)	5 (1.3)
Total	242 (63.0)	142 (37.0)	384 (100)



Graph 1: Age and Sex Distribution of Suspected Cases

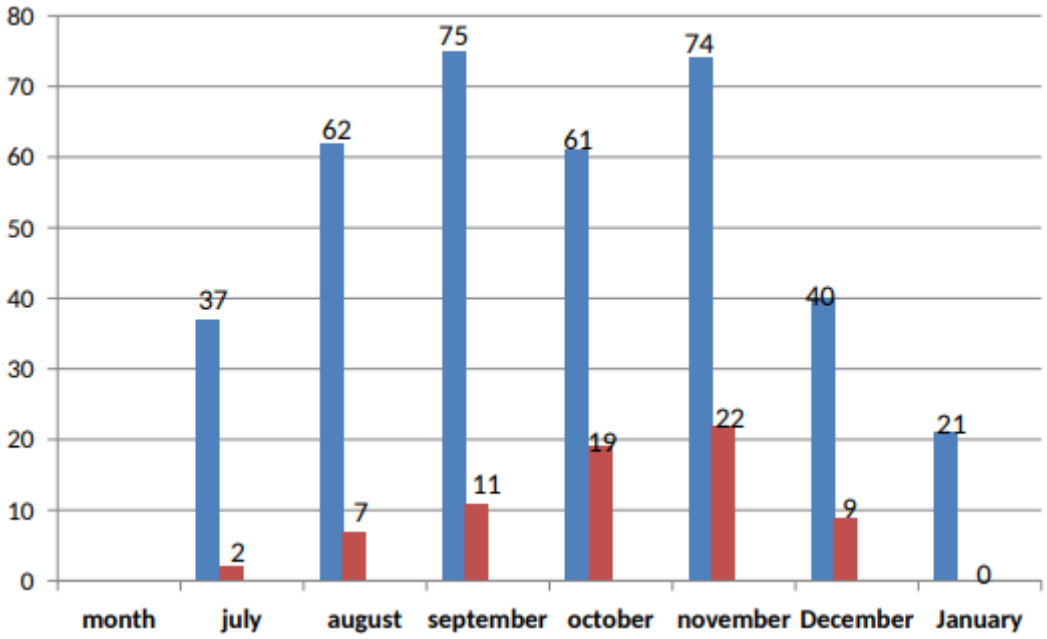
Graph 1 illustrates the demographic skew, with males consistently outnumbering females across age groups, peaking in 21–30 years (73% male).

Temporal and Diagnostic Patterns

Dengue transmission peaked post-monsoon, with the highest suspected cases in September (n=75; 19.5%) and November (n=74; 19.3%). Serology (NS1/IgM ELISA) confirmed 70 dengue-positive cases (18.2% of suspected cases), with the highest positivity in November (n=22) and October (n=19). RT-PCR confirmed 27/33 NS1-positive samples (81.8% concordance).

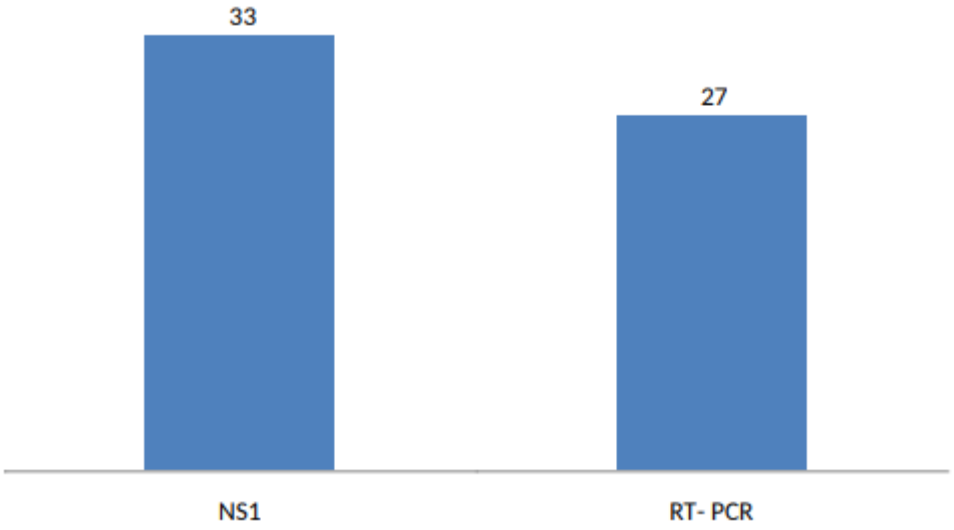
Table 2: Monthly Distribution of Suspected and Confirmed Dengue Cases

Month	Suspected Cases, n	NS1/IgM-Positive Cases, n
July	37	2
August	62	7
September	75	11
October	61	19
November	74	22
December	40	9
January	21	0
Total	384	70



Graph 2: Monthly Suspected vs. Confirmed Cases

Graph 2 highlights the surge in confirmed cases during October–November.



Graph 3: NS1 vs. RT-PCR Positivity

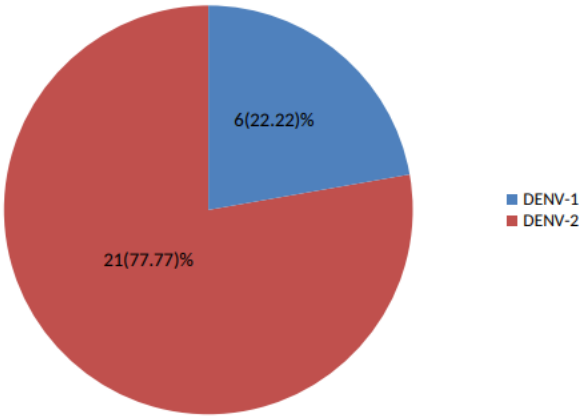
Graph 3 shows RT-PCR detected 27/33 NS1-positive samples.

Serotype Distribution and Clinical Correlates

Among the dengue cases analyzed, DENV-2 was the predominant serotype, accounting for 77.8% (n=21), while DENV-1 constituted 22.2% (n=6). The majority of affected individuals were males (77.8%) and fell within the 15–30 year age group (51.9%). Geographically, Sambalpur district bore the highest burden of cases (40.7%), with DENV-2 being the dominant strain in Sambalpur (9 out of 11 cases), whereas DENV-1 was exclusively detected in Bargarh (3 out of 3 cases). Clinically, fever was present in all patients (100%), followed by headache (85.2%) and bleeding manifestations (63.0%). Notably, DENV-1 infections were associated with a higher incidence of splenomegaly (66.7% compared to 9.5% in DENV-2) and retroorbital pain (83.3% vs. 28.6%). Thrombocytopenia was observed in 66.7% (n=18) of cases, with platelet counts falling below 50,000/µL, though no significant serotype-related differences were noted. Viral load, as indicated by Ct values, ranged from 12.22, representing the highest viral load, to 35.79, the lowest.

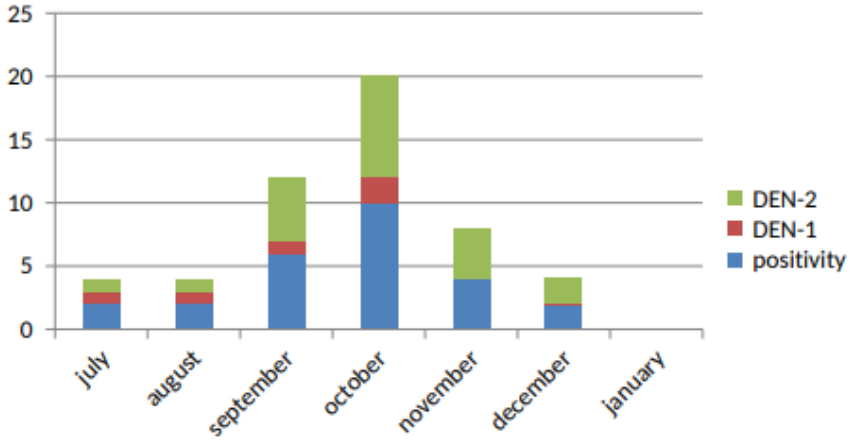
Table 3: Dengue Serotype Characteristics (n=27 RT-PCR-Positive Cases)

Parameter	**Total, n (%)**	**DENV-1, n (%)**	**DENV-2, n (%)**
Age Group (Years)			
0–15	2 (7.4)	0	2 (9.5)
15–30	14 (51.9)	4 (66.7)	10 (47.6)
30–45	8 (29.6)	2 (33.3)	6 (28.6)
≥45	3 (11.1)	0	3 (14.3)
Sex			
Male	21 (77.8)	4 (66.7)	17 (81.0)
Female	6 (22.2)	2 (33.3)	4 (19.0)
Peak Month			
October	10 (37.0)	2 (33.3)	8 (38.1)
Key Symptoms			
Bleeding manifestations	17 (63.0)	3 (50.0)	14 (66.7)
Splenomegaly	6 (22.2)	4 (66.7)	2 (9.5)
Platelets (/µL)			
<50,000	18 (66.7)	4 (66.7)	14 (66.7)
>100,000	6 (22.2)	1 (16.7)	5 (23.8)



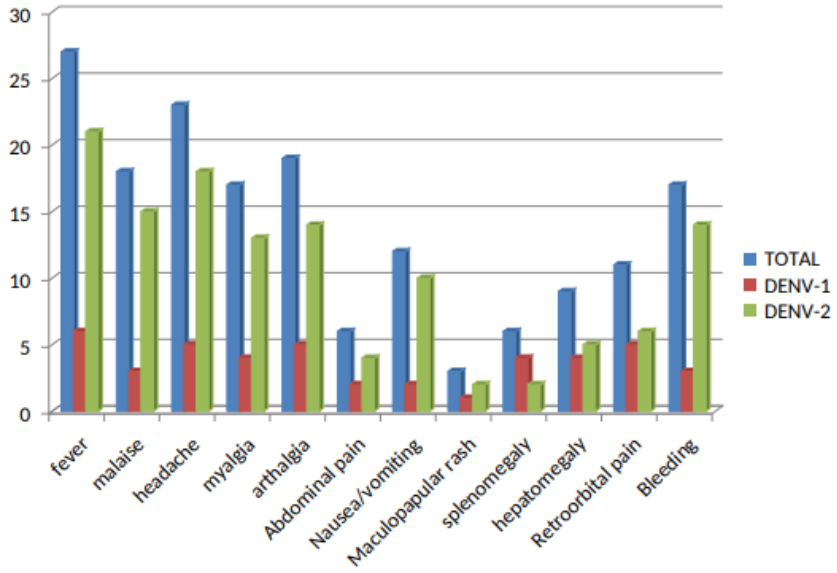
Graph 4: Serotype Distribution

Graph 4 confirms DENV-2 predominance.



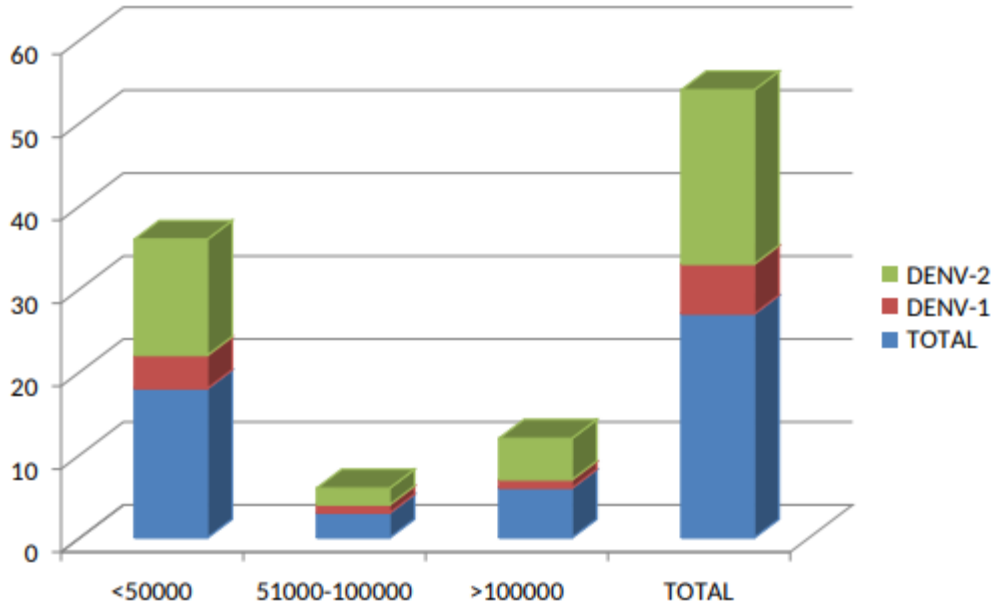
Graph 5: Monthly Serotype Trends

Graph 5 shows DENV-2 peaked in October.



Graph 6: Clinical Symptoms by Serotype

Graph 6 contrasts symptom profiles.



Graph 7: Platelet Counts by Serotype

Graph 7 reveals similar thrombocytopenia rates.

DENV-2 was the dominant serotype in Western Odisha, with peak transmission in October. Males and young adults (15–30 years) were most affected. Bleeding and thrombocytopenia were common, but splenomegaly and retroorbital pain were more frequent in DENV-1 infections. These findings underscore the need for serotype-specific surveillance and clinical management.

4. DISCUSSION

Dengue remains a public health problem of importance in the tropical and subtropical world, including India, where it is hyperendemic with all four serotypes circulating. The current study, undertaken between February 2021 and December 2022 in Western Odisha, yields information regarding the seroprevalence, molecular profile, and clinical pattern of dengue infection. The seropositivity in this research was 18.22% overall, which confirms results from other such studies throughout India. The increase in dengue has been reported to be caused by various factors including poor sanitation conditions, population growth through rapid urbanization, and predisposing conditions of mosquito breeding [9].

The incidence was higher among males with a ratio of 1.7:1 for males to females, as seen in other reports. A majority of the affected patients were in the age group 16–30 years, which implies that adult individuals with higher outdoor exposure are at greater risk [10]. Out of the 33 NS1-positive samples that were analyzed through multiplex real-time PCR, 27 (81.8%) were positive for dengue RNA. Two serotypes only, DENV-1 and DENV-2, were detected from among them, of which DENV-2 was more prevalent (77.77%). This pattern is in line with previous research in India, implying a changing serotype dominance over time [11,12].

Molecular confirmation revealed male predominance among RT-PCR positive cases (3.5:1), which may have resulted from greater mobility and outdoor exposure. Infections mainly during the post-monsoon period, especially between the months of September to November, depicted seasonal transmission patterns consistent with heightened mosquito activity [13]. The most frequent symptom clinically was fever, followed by headache, arthralgia, and myalgia. Nausea, vomiting, hepatomegaly, and splenomegaly were also observed, the bleeding manifestations being predominantly observed in the cases of DENV-2 [14].

Platelet counts were significantly low in most patients, with 66% having levels <50,000 cells/mm³, validating the correlation of thrombocytopenia with dengue severity [15]. In totality, the study underscores the significance of early diagnosis by employing both serological and molecular techniques to detect circulating serotypes, determine seasonal patterns, and track clinical outcomes essential in surveillance and management of disease.

5. CONCLUSION

The current study isolated the occurrence of two dengue virus serotypes—DENV-2 as the dominant one, followed by DENV-1—of Western Odisha. The results highlight the significance of adopting both serological and molecular diagnostic methods for efficient surveillance and early detection. Although serology is commonly employed, it is probable to be less sensitive

when viral load is low. Conversely, molecular techniques like RT-PCR provide better precision and dependability, enabling qualitative and quantitative estimation of infection, including disease severity. With the seasonal pattern of dengue transmission, particularly after and during monsoon, prompt preventive and long-term serosurveillance are needed to control outbreaks and mitigate disease burden in endemic areas.

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