

Evaluating the Efficacy of Serodiagnosis Technique of Dengue Virus Infection: An Epidemiological Study and Surveillance Analysis in Dimapur, Nagaland, India

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Abstract: *This study assessed the serodiagnostic and epidemiological characteristics of dengue virus (DENV) infection in Dimapur, Nagaland, India. A prospective study was conducted at the Department of Microbiology, District Hospital, Dimapur, from March 2023 to February 2024. Serum samples from 7,074 patients with suspected dengue infection were screened using rapid diagnostic tests for NS1 antigen and IgM antibodies, followed by NS1 and IgM ELISA confirmation. Selected samples were analyzed by RT-PCR for DENV serotyping. ELISA confirmed dengue infection in 2,010 cases, including 1,461 NS1-positive and 549 IgM-positive samples. Cases increased during the post-monsoon period, with the highest number recorded in October. Infection was more common among males and was most frequent in individuals aged 21–30 years. Among 39 samples suitable for molecular analysis, DENV-2 was detected in 13 samples, while 10 demonstrated co-infection with DENV-1 and DENV-2. Environmental assessment identified water logging, swamps, and mosquito breeding sites as potential contributors to transmission. The findings indicate substantial dengue transmission in Dimapur, characterized by seasonal and demographic variation and circulation of multiple DENV serotypes. Strengthening timely serological diagnosis, molecular surveillance, and integrated vector-control measures, particularly during the post-monsoon season, is essential for effective dengue prevention and control.*

Keywords: Dengue virus, ELISA, DENV, Serotyping, Epidemiology.

1. Introduction

Dengue is a major public health concern in tropical and subtropical regions and has expanded geographically in recent decades. Rapid urbanization, population growth, human mobility, environmental and climatic changes, and inadequate vector control have contributed to its continued transmission^{1,2,3}. India is endemic for dengue, with outbreaks reported across several states and increasing transmission in urban, rural, and peri-urban areas⁴. The first virologically confirmed outbreak in India was reported during 1963–1964 in Calcutta and along the eastern coast⁵. Since then, dengue has become an important cause of acute febrile illness and hospitalization. Despite improvements in surveillance and laboratory diagnosis, the true disease burden may be underestimated because of asymptomatic or mild infections, variations in healthcare-seeking behaviour, and limitations in diagnostic and reporting systems⁶. Dengue presents with a broad clinical spectrum, ranging from asymptomatic infection and febrile illness to dengue with warning signs and severe dengue. Because its clinical manifestations may overlap with other febrile illnesses, laboratory confirmation is important for diagnosis, patient management, outbreak detection, and epidemiological surveillance^{1,7}. During the early viraemic phase, dengue viral RNA and non-structural protein 1 (NS1) antigen can be detected, whereas serological tests identify the host antibody response. Dengue-specific IgM is commonly used as an indicator of recent infection, while IgG indicates previous exposure and may help distinguish primary from secondary infection when interpreted with clinical and laboratory

findings^{7,8}. Serological sensitivity is influenced by the timing of specimen collection, assay type, and infection status. Cross-reactivity with antibodies to other flaviviruses may also affect interpretation^{8,9}. Accurate and timely diagnosis is essential for effective dengue surveillance and for understanding local epidemiological patterns. Dimapur, an important urban centre of Nagaland, has environmental and demographic conditions that may support mosquito-borne disease transmission. However, locally generated data on dengue serodiagnosis and its epidemiological characteristics remain limited. Therefore, the present study was undertaken to evaluate the serodiagnostic findings of dengue virus infection and assess the epidemiological and surveillance characteristics of dengue cases in Dimapur, Nagaland, India. The findings may provide locally relevant evidence to support dengue diagnosis, surveillance, and public-health planning.

2. Objective

- 1) To study the transmission dynamics of dengue virus, including vector interaction, environmental factor and human behaviour that influence the speed of the virus.
- 2) To investigate the connection between a patient's clinical presentation and their serological findings using igm&ns1 elisa serological test

3. Materials and Methods

Study Design and Setting

A prospective observational study was conducted in the Department of Microbiology, District Public Health Laboratory, Dimapur, Nagaland, India, from March 2023 to February 2024. Patients clinically suspected of dengue infection presenting within the early phase of illness were enrolled after obtaining informed consent. A total of 7,074 suspected dengue cases were included in the study. Patients presenting with fever and at least two compatible clinical or laboratory features, including nausea, vomiting, rash, headache, myalgia /arthralgia, positive tourniquet test, leukopenia, or warning signs, were considered clinically suspected cases. Patients referred from private hospitals, clinics, and laboratories were also included.

1) Specimen Collection and Processing

Approximately 2–5 mL of venous blood was collected aseptically from each participant. Serum was separated by centrifugation and processed for dengue NS1 antigen and anti-dengue IgM/IgG antibody detection. Samples requiring confirmatory testing were stored in aliquots at -80°C until further analysis. Relevant demographic and clinical information, including age, sex, date of onset of symptoms, presenting symptoms, and laboratory findings, was recorded using a structured data-collection proforma.

2) Rapid Serological Testing

Dengue NS1 antigen and anti-dengue IgM/IgG antibodies were initially screened using commercially available rapid immunochromatographic assays, including the Agappe and Abbott-Bioline dengue rapid test kits, according to the respective manufacturers' instructions. The assays qualitatively detect dengue NS1 antigen and/or anti-dengue IgM and IgG antibodies in serum, plasma, or whole blood. Results were interpreted within the manufacturer's specified time frame. Multiple trained laboratory personnel independently interpreted the test results to minimize observer bias.

3) ELISA

Rapid-test-positive samples were subjected to confirmatory enzyme-linked immunosorbent assay (ELISA). Dengue-specific IgM was detected using the NIV Dengue IgM Capture ELISA, while dengue NS1 antigen was detected using the Erba Lisa Dengue NS1Ag ELISA, following the respective manufacturer's protocols. For IgM detection, patient IgM antibodies were captured using anti-human IgM-coated wells and subsequently reacted with dengue antigen and enzyme-labelled detection antibodies. For NS1

detection, dengue NS1 antigen present in the specimen was captured by monoclonal anti-NS1 antibodies and detected using enzyme-labelled antibodies. Optical density was measured at 450 nm using an ELISA microplate reader. A sample was considered laboratory-confirmed for dengue when either dengue-specific IgM antibody or NS1 antigen was detected by ELISA, in accordance with the study case definition.

4) Dengue Virus Serotyping

Virus strains and clinical samples

DENV strains representing the four serotypes (DENV-1, DENV-2, DENV-3, and DENV-4), Serum samples from clinically suspected dengue patients were used for molecular confirmation and serotyping. RNA extraction and cDNA synthesis. Viral RNA was extracted from 140 μL of patient serum/plasma using the QIAamp Viral RNA Mini Kit (QIAGEN, Cat. No. 52906), according to the manufacturer's instructions. Briefly, samples were lysed with AVL buffer containing carrier RNA, followed by ethanol addition, silica-membrane purification, washing, and elution in AVE buffer. Extracted RNA was stored at -80°C until further analysis. Complementary DNA (cDNA) was synthesized from the extracted RNA using a reverse-transcription system with random primers, following the manufacturer's recommended protocol. The resulting cDNA was used as the template for nested reverse-transcription polymerase chain reaction (RT-PCR). Nested RT-PCR for DENV serotyping. DENV serotyping was performed using a two-round nested RT-PCR assay. The first round employed conserved dengue primers D1 and D2, followed by a second, serotype-specific amplification using primer D1 and one of the type-specific primers (TS1–TS4) corresponding to DENV-1, DENV-2, DENV-3, and DENV-4, respectively.

PCR amplification was performed according to the established thermal cycling protocol, with an initial denaturation followed by 35 cycles of denaturation, annealing, and extension, and a final extension. The expected amplicon sizes were approximately 482 bp for DENV-1, 199 bp for DENV-2, 290 bp for DENV-3, and 389 bp for DENV-4. A 511-bp product was expected from the first-round amplification.

PCR products were separated by electrophoresis on a 1.5% agarose gel and visualized using an appropriate DNA-binding stain under UV illumination. Serotypes were identified by comparing the observed band sizes with a molecular weight marker and the expected serotype-specific amplicon sizes.

Table 1: Oligonucleotide primers for DENV serotype

Primary	Nucleotide Sequence(5' to 3')	Position	Product size(bp)
D1	TCA ATA TGC TGA AAC GCG CGA GAA ACC G	134-161	511(D1 & D2)
D2	TTGCACCAACAGTCAATGTCTTCAGGTC	616-644	511(D1 & D2)
TS1	CGT CTC AGT GAT CCG GGG C	568-586	482(D1 & TS1)
TS2	CGC CAC AAG GGC CAT GAA CAG	232-252	199(D1 & TS2)
TS3	TAA CAT CAT CAT GAG ACA GAG C	400-421	290(D1 & TS3)
TS4	TGT TGT CIT AAA CAA GAG AGC TC	506-527	389(D1 & TS4)

5) Agarose Gel Electrophoresis

PCR products were analyzed by electrophoresis on 1.5% agarose gels prepared in TAE buffer. A DNA molecular-weight ladder, positive control, negative control, and PCR products were loaded into separate wells. Electrophoresis was performed under appropriate voltage conditions, and DNA bands were visualized under UV illumination. The presence of bands at the expected molecular sizes was interpreted as evidence of the corresponding DENV serotype.

6) Supportive Laboratory Investigations

Routine hematological investigations, including complete blood count (CBC) and erythrocyte sedimentation rate (ESR), were performed where indicated. Blood samples were collected using standard aseptic procedures. EDTA-anticoagulated blood was used for hematological analysis, while serum samples collected in plain tubes were used for serological and/or molecular investigations. Laboratory investigations were performed using standard operating procedures and manufacturer's instructions for the respective instruments and reagents.

7) Peripheral Blood Smear

Peripheral blood smears were prepared from EDTA-anticoagulated blood and stained with Leishman stain. The smear was examined microscopically under the oil-immersion objective for differential leukocyte counting and assessment of red and white blood cell morphology. At least 100 leukocytes were counted for DLC.

4. Results and Discussion

A total of 7,074 suspected dengue cases were included from March 2023 to February 2024. Cases showed seasonal variation, with a marked increase during the post-monsoon period and the highest number recorded in October. Males and the 21–30-year age group were predominantly affected. By RDT, 4,340 cases were NS1-positive and 2,734 were IgM-positive, while confirmatory ELISA identified 2,010 positive samples, including 1,461 NS1-positive and 549 IgM-positive samples. NS1 ELISA results from 40 cross-checked samples were concordant with the initial findings.

For molecular characterization, 39 of 40 selected samples were successfully tested by PCR. Twenty-three were PCR-positive, including 13 DENV-2 and 10 DENV-1/DENV-2 co-infections, indicating DENV-2 as the predominant circulating serotype. IgG Capture ELISA demonstrated both primary and secondary dengue infections.

Fever, headache, myalgia, and rash were the most common clinical manifestations, while severe bleeding, coma, and shock were uncommon. Cases were clustered mainly in Ward 12 and Signal Angami in Dimapur Sadar, with scattered cases in Medziphema, Dhansiripar, and Niuland. The predominance among males and young adults may reflect increased exposure to mosquito habitats and outdoor activities.

Overall, 44.56% of evaluated samples were positive by the diagnostic methods used. The findings highlight the predominance of NS1 positivity, circulation of DENV-2,

and occurrence of DENV-1/DENV-2 co-infections and secondary infections. These results emphasize the importance of combining serological and molecular surveillance for effective dengue diagnosis, monitoring, and control in Dimapur, Nagaland.

Table 2: Laboratory Investigation Findings among Dengue-Positive Cases

Laboratory investigation	Category	Number (n)	Percentage (%)
RDT (n = 7,074)	NS1-positive	4,340	61.35
	IgM-positive	2,734	38.65
Confirmatory ELISA (n = 2,010)	NS1-positive	1,461	72.69
	IgM-positive	549	27.31
NS1 ELISA cross-check (n = 40)	Concordant	40	100
PCR (n = 39 tested)	PCR-positive	23	58.97
	PCR-negative	16	41.03
PCR serotyping (n = 23 positive)	DENV-2	13	56.52
	DENV-1 + DENV-2 co-infection	10	43.48

5. Conclusion

This study highlights the epidemiological and diagnostic characteristics of dengue in Dimapur, Nagaland, during March 2023–February 2024. Dengue transmission showed a clear seasonal pattern, with cases increasing during the monsoon and post-monsoon periods and peaking in October. Young adults, particularly those aged 21–30 years, and males constituted a larger proportion of diagnosed cases.

The study also demonstrated the importance of combining rapid diagnostic tests with confirmatory ELISA and molecular methods for reliable dengue detection and surveillance. Molecular testing identified DENV-2 and DENV-1/DENV-2 co-infections, emphasizing the need for continued serotype surveillance.

The findings support strengthening seasonal surveillance, early diagnosis, targeted vector control, environmental management, and community-based preventive measures, particularly in areas with higher case clustering. Continued molecular and epidemiological surveillance is warranted to monitor changes in dengue serotypes and transmission patterns and to guide timely public health interventions in Dimapur and surrounding regions.

6. Future Scope

This study was limited by the small number of samples selected for PCR and the relatively short study period, which may not fully represent dengue serotype diversity. Compared with previous or historical studies based mainly on clinical diagnosis or serological testing, the present study provides a more comprehensive approach by combining RDT, ELISA, IgG testing, and molecular PCR-based serotyping. It identified DENV-2 predominance, DENV-1/DENV-2 co-infection, and both primary and secondary infections. Future research should include larger sample sizes, multi-year surveillance, wider geographical coverage, genomic sequencing, and integration of climatic and vector data to improve dengue surveillance and control.

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