Section: Miscellaneous



Original Research Article

EVALUATION OF DIAGNOSTIC STRATEGIES FOR DENGUE FEVER: A RETROSPECTIVE STUDY FROM A HOSPITAL IN SOUTH INDIA

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ABSTRACT

Background: Dengue fever poses a significant global health burden, with India contributing a substantial proportion of cases. Accurate and timely diagnosis is critical for disease management and intervention.

Materials and Methods: A retrospective study was conducted in a regional hospital from November 2021 to September 2024. Patients suspected of dengue fever underwent NS1 antigen testing, and IgM antibody testing was performed on NS1-negative cases with persistent symptoms. Data were analyzed using the R software.

Results: Of the 10,606 samples tested, 826 were NS1 positive (7.8%), with males (8.5%) showing slightly higher positivity than females (7.1%). The highest positivity was in the 19-30 age group (12.4%) and during the monsoon season (20%). In follow-up cases, IgM testing showed a positivity rate of 26.9% (n=836), with 225 positive cases.

Conclusion: Combining NS1 antigen and IgM antibody testing significantly enhances dengue diagnosis, ensuring timely management. Regional and demographic trends highlight the importance of targeted interventions during high-risk periods.

Keywords: Dengue, NS1 antigen, IgM antibody, diagnostic yield, retrospective study, public health.

INTRODUCTION

Dengue fever remains a major global health concern, with over 10 million cases and 6,508 deaths reported worldwide in 2024, marking the highest recorded numbers to date.[1] India accounts for nearly 34% of dengue cases, highlighting the importance of local data in improving diagnostic and management strategies.^[2] Despite the high prevalence of the disease, regional data remain limited, hindering the development of targeted interventions.[3] Public health surveillance systems in India are often inadequate, missing mild febrile illnesses and subclinical infections, which account for about 75% of dengue cases. Furthermore, a large portion of the population seeks care in the private healthcare sector, where data collection is insufficient, leading to significant under-reporting.[4] As a result. mathematical models estimate that the actual number of dengue cases in India may be up to 282-fold higher than reported by the official surveillance system.^[5] Dengue fever is caused by the dengue virus, a positive-sense RNA virus belonging to the genus Flavivirus within the family Flaviviridae. [6] Initially, dengue was associated with four antigenically related serotypes (DENV-1, DENV-2, DENV-3, and DENV-4). However, a fifth variant, DENV-5, was identified in October 2013.^[7] The primary vectors of dengue transmission are Aedes mosquitoes, particularly Aedes aegypti and Aedes albopictus. In tropical regions, peak transmission occurs during the rainy season,^[7] when water collection sites such as discarded tyres, coolers, old pots, and coconut shells provide ideal breeding grounds for mosquitoes. During this period, mosquito density increases significantly, with an average of 3-4 female mosquitoes per household compared to 1-2 during the dry season.[8]

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Diagnostic methods such as virus isolation through cell culture and nucleic acid detection via reverse transcriptase polymerase chain reaction (RT-PCR) are accurate but require significant time and expensive laboratory infrastructure. [6] Consequently, in resource-limited settings, detecting NS1 antigen and virus-specific IgM/IgG antibodies serves as crucial diagnostic tools.^[9] NS1 antigen testing is helpful in the early stages of dengue infection. In primary infections, the NS1 antigen can be detected as early as Day 1 of fever, reaches its peak between Days 1 and 5, and remains detectable for up to 18 days.^[10] In secondary infection, the NS1 antigen also appears around Day 1 of fever, peaks between Days 1 and 5, and persists for up to 18 days. As a result, NS1 antigen testing is recommended during the first 5 days of fever to assist in diagnosing acute dengue infection. [11,12]

For IgM antibody testing, the detection timeline varies between primary and secondary infections. In primary infection, IgM antibodies are typically detectable around Day 5 of fever, peak approximately two weeks after symptom onset, and remain detectable for up to 90 days. In secondary infection, IgM antibodies appear earlier, usually around Days 2 to 3 of fever, but their increase is more modest, and they tend to disappear more quickly.^[13] Therefore, IgM antibody testing is most useful after Day 5 of fever, particularly when the NS1 test is negative, to help confirm a diagnosis of dengue. IgG antibodies are typically detectable between 14 to 21 days after symptom onset in primary dengue infections, gradually increasing and remaining detectable for life. In secondary infection, IgG antibodies are already present due to prior exposure, with a rapid rise observed around Days 4-5, and they remain detectable for life. [14] Since no specific treatment or vaccine for dengue exists, effective prevention and control rely on epidemiological surveillance. Such surveillance provides accurate disease burden estimates, aiding in implementing targeted vectorcontrol measures.^[15]

This study aims to analyse demographic trends, identify seasonal patterns, and evaluate the diagnostic performance of NS1 antigen and IgM antibody testing in a secondary-level hospital through a retrospective approach.

MATERIALS AND METHODS

This retrospective hospital-based study was conducted to evaluate the burden of dengue and assess the diagnostic strategies employed at a regional hospital. The study utilized anonymised hospital records data covering patient demographics, sub-district of origin, and diagnostic test results. These records were systematically analyzed to explore gender distribution, age groups affected, geographic patterns, and temporal trends in dengue cases, offering a comprehensive understanding of the disease burden. Patients with fever lasting three or more days were subjected to initial diagnostic testing for the NS1 antigen using an ELISA kit (J. Mitra & Co. Pvt. Ltd., New Delhi). Patients with positive NS1 results were promptly documented and managed according to standard treatment protocols. For patients whose NS1 antigen test results were negative but continued to experience fever, a follow-up sample was tested for IgM antibodies using the MAC ELISA (NIV Pune) to confirm or rule out dengue infection. The diagnostic approach was designed to maximize sensitivity and ensure accurate detection of dengue in both early and later stages of the disease. Data analysis was performed using R Studio (version 2024.04.2+764) with packages such as 'readxl,' 'dplyr,' 'ggplot2,' 'tidyverse,' and 'lubridate' for management, statistical analysis, visualization. Ethical clearance was not required for the study, as it utilized anonymized patient data from hospital records without direct patient involvement.

RESULTS

A total of 10,606 samples were tested for NS1 antigen between November 2021 and September 2024, of which 826 were positive, resulting in a positivity rate of 7.8%. Of the total samples analyzed, 5188 (48.9%) were males, and 5418 (51.1%) were females. The confirmed dengue cases among males were 441, with a positivity rate of 8.5%, while among females, there were 385 confirmed cases, resulting in a positivity rate of 7.1%. The highest positivity rate (12.4%) was observed in the 19–30 age group, with 275 cases out of 2220 samples.

Table 1: Sociodemographic distribution of Cases Analyzed in a Hospital-Based Retrospective Study (November 2021 to September 2024, N=10,606)

Sociodemographic Distribution	Total Samples Analyzed	Confirmed Cases	Positivity Percentage (%)
Gender			
Males	5188	441	8.5
Females	5418	385	7.1
Age			
0-18	2957	233	7.9
19-30	2220	275	12.4
31-40	1434	111	7.7
41-50	1296	86	6.6
51-60	1173	57	4.9
61+	1522	64	4.2
Total	10606		

In this study, a total of 5408 samples were collected from the outpatient department, of which 385 cases were confirmed, yielding a positivity rate of 7.1%. In comparison, the inpatient department had 5198 samples analyzed, with 441 confirmed cases, resulting in a higher positivity rate of 8.5%.

Further analysis of the sub-districts revealed that the majority of the samples were from Karaikal Taluk, where 8227 samples were analyzed, and 575 cases were confirmed, giving a positivity rate of 7.0%. In contrast, Thirunallar Taluk reported 1384 samples, with 177 confirmed cases, resulting in the highest positivity rate of 12.8%. Other sub-districts such as Nagapattinam (6.6%), Mayiladuthurai (13.8%), Tarangambadi (7.4%), and Nannilam (9.4%) had comparatively fewer samples tested, with varying positivity percentages.

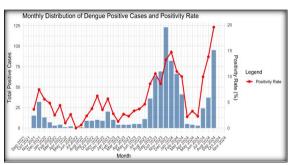


Figure 1: Month wise distribution of dengue cases

For the period between August 2023 and September 2024, 6,035 samples were tested for NS1, and 659 samples were positive, showing a positivity rate of 10.9%.

Additionally, during the same period (August 2023 - September 2024), 836 samples were tested for IgM antibodies, of which 225 samples were positive, resulting in a positivity rate of 26.9%.

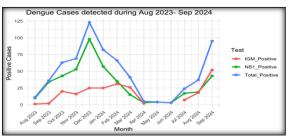


Figure 2: Impact of Combined NS1 and IgM Testing - Total Yield

DISCUSSION

Dengue fever is a significant public health concern, particularly in India, where the tropical climate and urbanisation foster ideal conditions for mosquito breeding. [16] This study highlights the demographic, seasonal, and geographic factors influence dengue transmission, underscoring the need for targeted public health strategies.

The highest positivity rate in the 19–30 age group observed in this study aligns with prior findings that

young adults are more likely to engage in outdoor activities, increasing their exposure to Aedes mosquito bites.^[16,17] Additionally, the higher positivity rate among males in this study reflects global patterns and may be attributed to their greater involvement in outdoor work and behavioural factors.^[18] These findings emphasise the importance of awareness campaigns and preventive programs targeting these high-risk groups.

The seasonal rise in dengue cases during the monsoon period highlights the strong link between rainfall and mosquito breeding. Similar observations have been documented, reinforcing the importance of strengthening vector control efforts before and during the monsoon season.^[20] Measures such as eliminating stagnant water, using insect repellents, and wearing protective clothing should be prioritised. Geographic variations in positivity rates, suggest that interventions should be tailored to local conditions. Targeted strategies, including community engagement and improved water management, can help reduce dengue transmission in these areas.

Combining NS1 antigen and IgM antibody testing significantly enhanced case detection in this study. While NS1 antigen testing is effective for early detection, IgM antibody testing identifies cases in later stages, providing a more comprehensive diagnostic approach. From August 2023 to September 2024, the addition of IgM antibody testing increased diagnostic sensitivity, with a positivity rate of 26.9% compared to 10.9% for NS1 testing alone. This combination aligns with findings from other studies that highlight the complementary roles of these tests in improving diagnostic accuracy. [21] Given its cost-effectiveness and practicality, this approach is valuable for improving dengue surveillance and ensuring timely clinical interventions.^[22]

Advancements in molecular diagnostics, such as RT-PCR, offer high sensitivity and specificity for detecting dengue. However, these methods are often costly and less accessible in resource-limited settings. [23, 24] Combining NS1 and IgM, testing provides an affordable and effective alternative. Studies have shown that combining diagnostic methods significantly improves sensitivity, with NS1 antigen and IgM antibody tests yielding a diagnostic performance comparable to molecular techniques like PCR. [23] This comprehensive diagnostic approach and context-specific public health strategies are essential for addressing the ongoing challenge of dengue in high-burden regions.

Limitations

This study has a few limitations. The timing of sample collection, about symptom onset was not consistently recorded, which may have affected the accuracy of the results. Future research should address this by systematically documenting sample collection timing. IgG testing was not included, making the distinction between primary and secondary dengue infection difficult. It relied on existing records as a retrospective study, which may

have gaps or inconsistencies. Prospective studies with planned data collection are needed to address this. The findings are based on data from a single hospital, so they may not fully represent other regions with different conditions. Despite these limitations, this study offers important insights into dengue transmission and highlights cost-effective diagnostic strategies that can guide better public health interventions in high-burden regions.

CONCLUSION

This study highlights the importance of combining NS1 antigen and IgM antibody testing to enhance dengue detection, particularly during monsoon seasons when cases surge. The integrated diagnostic approach ensures no infections are missed, facilitating timely clinical interventions and improving patient outcomes. The findings underscore the need for targeted public health strategies in highburden regions, informed by demographic, seasonal, and geographic patterns of dengue transmission. Addressing challenges such as incomplete documentation of sample collection timing and incorporating IgG testing would further refine accuracy and enhance diagnostic surveillance. These improvements are critical for mitigating the burden of dengue and improving outcomes in affected populations.

REFERENCES

- Deng J, Zhang H, Wang Y, Liu Q, Du M, Yan W, et al. Global, regional, and national burden of dengue infection in children and adolescents: an analysis of the Global Burden of Disease Study 2021. eClinicalMedicine [Internet]. 2024 Dec 1 [cited 2025 Mar 8];78. Available from: https://www.thelancet.com/journals/eclinm/article/PIIS2589-5370(24)00522-4/fulltext?rss=yes
- Dutta O, Prasanth A, Kumari A, Akanksha K, Deeba F, Salam N. Burden of dengue, leishmaniasis and lymphatic filariasis in India and its states from 1990–2019: Analysis from the Global Burden of Disease study (GBD 2019). Panda AK, editor. PLOS ONE [Internet]. 2023 Oct 18 [cited 2025 Apr 2];18(10):e0292723. Available from: https://dx.plos.org/10.1371/journal.pone.0292723
- Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature. 2013 Apr 25;496(7446):504–7.
- Ganeshkumar P, Murhekar MV, Poornima V, Saravanakumar V, Sukumaran K, Anandaselvasankar A, et al. Dengue infection in India: A systematic review and meta-analysis. Rodriguez-Barraquer I, editor. PLoS Negl Trop Dis [Internet]. 2018 Jul 16 [cited 2025 Mar 6];12(7):e0006618. Available from: https://dx.plos.org/10.1371/journal.pntd.0006618
- Shepard DS, Halasa YA, Tyagi BK, Adhish SV, Nandan D, Karthiga KS, et al. Economic and disease burden of dengue illness in India. Am J Trop Med Hyg. 2014 Dec;91(6):1235– 42
- World Health Organisation. Dengue guidelines, for diagnosis, treatment, prevention and control. 2009th ed. Geneva, Switzerland: World Health Organization;
- Huber K, Le Loan L, Hoang TH, Tien TK, Rodhain F, Failloux AB. Aedes aegypti in south Vietnam: ecology, genetic structure, vectorial competence and resistance to insecticides. Southeast Asian J Trop Med Public Health. 2003 Mar;34(1):81-6.

- 8. Zumla A. Mandell, Douglas, and Bennett's principles and practice of infectious diseases [Internet]. Vol. 10. 2010 [cited 2025 Jan 12]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7128814/
- Liu LT, Dalipanda T, Jagilly R, Wang YH, Lin PC, Tsai CY, et al. Comparison of two rapid diagnostic tests during a large dengue virus serotype 3 outbreak in the Solomon Islands in 2013. PLoS ONE [Internet]. 2018 Aug 10 [cited 2025 Jan 12];13(8):e0202304. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6086442/
- Anand AM, Sistla S, Dhodapkar R, Hamide A, Biswal N, Srinivasan B. Evaluation of NS1 Antigen Detection for Early Diagnosis of Dengue in a Tertiary Hospital in Southern India. J Clin Diagn Res JCDR [Internet]. 2016 Apr [cited 2025 Jan 29];10(4):DC01–4. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4866096/
- Chan HBY, How CH, Ng CWM. Definitive tests for dengue fever: when and which should I use? Singapore Med J [Internet]. 2017 Nov [cited 2025 Jan 29];58(11):632–5.
 Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5691225/
- Changal KH, Raina AH, Raina A, Raina M, Bashir R, Latief M, et al. Differentiating secondary from primary dengue using IgG to IgM ratio in early dengue: an observational hospital based clinico-serological study from North India. BMC Infect Dis. 2016 Nov 28;16(1):715.
- Shu PY, Huang JH. Current advances in dengue diagnosis. Clin Diagn Lab Immunol. 2004 Jul;11(4):642–50.
- 14. Blacksell SD, Jarman RG, Bailey MS, Tanganuchitcharnchai A, Jenjaroen K, Gibbons RV, et al. Evaluation of Six Commercial Point-of-Care Tests for Diagnosis of Acute Dengue Infections: the Need for Combining NS1 Antigen and IgM/IgG Antibody Detection To Achieve Acceptable Levels of Accuracy. Clin Vaccine Immunol [Internet]. 2011 Dec [cited 2025 Mar 8];18(12):2095–101. Available from: https://journals.asm.org/doi/10.1128/cvi.05285-11
- Murray NEA, Quam MB, Wilder-Smith A. Epidemiology of dengue: past, present and future prospects. Clin Epidemiol [Internet]. 2013 Aug 20 [cited 2025 Jan 29];5:299–309. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3753061/
- 16. World Health Organisation. Dengue and severe dengue [Internet]. WHO Fact sheet. 2024 [cited 2025 Feb 26]. Available from: https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue
- Mishra S, Ramanathan R, Agarwalla SK. Clinical Profile of Dengue Fever in Children: A Study from Southern Odisha, India. Scientifica. 2016;2016:6391594.
- Shah GS, Islam S, Das BK. Clinical and laboratory profile of dengue infection in children. Kathmandu Univ Med J KUMJ. 2006;4(1):40–3.
- Rao C, Kaur H, Gupta N, Sabeena SP, Ambica R, Jain A, et al. Geographical distribution of primary & secondary dengue cases in India - 2017: A cross-sectional multicentric study. Indian J Med Res. 2019 Apr;149(4):548–53.
- Reiter P. Climate change and mosquito-borne disease. Environ Health Perspect. 2001 Mar;109 Suppl 1(Suppl 1):141–61.
- Anand AM, Sistla S, Dhodapkar R, Hamide A, Biswal N, Srinivasan B. Evaluation of NS1 Antigen Detection for Early Diagnosis of Dengue in a Tertiary Hospital in Southern India. J Clin Diagn Res JCDR. 2016 Apr;10(4):DC01-04.
- Runge-Ranzinger S, McCall PJ, Kroeger A, Horstick O. Dengue disease surveillance: an updated systematic literature review. Trop Med Int Health [Internet]. 2014 Sep [cited 2025 Feb 26];19(9):1116–60. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4253126/
- Kassim FM, Izati MN, TgRogayah T a. R, Apandi YM, Saat Z. Use of dengue NS1 antigen for early diagnosis of dengue virus infection. Southeast Asian J Trop Med Public Health. 2011 May;42(3):562–9.
- 24. Chua KB, Mustafa B, Abdul Wahab AH, Chem YK, Khairul AH, Kumarasamy V, et al. A comparative evaluation of dengue diagnostic tests based on single-acute serum samples for laboratory confirmation of acute dengue. Malays J Pathol. 2011 Jun;33(1):13–20.