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MOLECULAR CHARACTERIZATION AND EPIDEMIOLOGY OF DENGUE VIRUS IN CENTRAL INDIA: INSIGHTS INTO SEROTYPE DISTRIBUTION AND EVOLUTIONARY TRENDS

Rashmi Ranjan Rout[1], Dr. Debasish Samal[2], Dr. Monalisa Subudhi[3], Dr. V.K. Ramnani[4],

[1]Ph.D. Research Scholar LNCT Medical College, Bhopal Email: micro.rashmi@gmail.com
[2]Assistant Professor LtBRKM Medical College, Jagdalpur
[3]Associate Professor SOA Medical College, Bhubaneswar
[4]*Professor & Head LNCT Medical College, Bhopal

***Correspondent Author:** Dr. V.K. Ramnani
*Professor & Head LNCT Medical College, Bhopal

Abstract
Dengue virus (DENV) is a significant public health concern, particularly in tropical and subtropical regions where it causes a wide range of clinical manifestations, from mild febrile illness to severe forms like dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). This study focuses on the molecular characterization of DENV strains circulating in Central India, an area prone to frequent dengue outbreaks. By analyzing genomic data from clinical samples, we aimed to elucidate the serotype distribution, genetic diversity, and evolutionary patterns of DENV in this region. The study also explores the epidemiological implications of these molecular characteristics, providing valuable insights into local transmission dynamics and public health challenges. The findings highlight the critical role of continuous molecular surveillance in informing effective dengue control strategies.

Keywords: Dengue virus, Molecular characterization, Serotype distribution, Central India, Epidemiology, Evolutionary trends

1. Introduction

Dengue fever, caused by the Dengue virus (DENV), represents a significant and growing public health challenge, particularly in tropical and subtropical regions. The World Health Organization (WHO) estimates that around 390 million dengue infections occur globally each year, with approximately 96 million manifesting clinically (Bhatt et al., 2013). The disease is primarily transmitted by *Aedes* mosquitoes, notably *Aedes aegypti* and *Aedes albopictus*, and involves four antigenically distinct serotypes: DENV-1, DENV-2, DENV-3, and DENV-4 (Guzman & Harris, 2015). Each serotype can cause a spectrum of clinical outcomes, from asymptomatic infection to severe conditions such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), which can be fatal without timely medical intervention (Halstead, 2007).

Central India, characterized by its tropical climate and seasonal monsoons, is a hyperendemic region for dengue. The region experiences frequent outbreaks, driven by the proliferation of mosquito vectors during the rainy season and the dense human populations that provide a continuous host reservoir for the virus (Gubler, 2012). The molecular characterization of circulating DENV strains is crucial for

understanding the epidemiology of the disease in this region. Such studies provide insights into the genetic diversity and evolutionary trends of the virus, which are essential for developing effective public health interventions, including vector control strategies and vaccine development (Messina et al., 2014).

Molecular surveillance of DENV is particularly important in hyperendemic areas like Central India, where the co-circulation of multiple serotypes can lead to increased disease severity due to antibody-dependent enhancement (ADE) (Dejnirattisai et al., 2010). Previous studies have shown that genetic variations within the DENV genome, particularly in the envelope (E) and non-structural (NS) genes, can influence viral virulence, transmission efficiency, and immune response (Rico-Hesse, 2010). This study aims to provide a comprehensive molecular characterization of DENV strains circulating in Central India, focusing on serotype distribution, genetic diversity, and evolutionary dynamics. The findings are expected to contribute to the understanding of dengue epidemiology in the region and to inform the design of targeted public health interventions.

2. Materials and Methods

2.1. Study Area and Population

The study was conducted in Central India, encompassing the states of Madhya Pradesh, Chhattisgarh, and parts of Maharashtra, which are known to be hyperendemic for dengue. These regions were selected due to their high incidence of dengue cases, particularly during the monsoon season when the *Aedes* mosquito population peaks. The study population included patients presenting with clinical symptoms of dengue at major tertiary care hospitals in these regions between November 2020 to October 2023 at J.K hospital, Bhopal in Central India. Ethical approval for the study was obtained from the institutional review boards of theparticipating hospitals, and written informed consent was collected from all participants or their legalguardians.

2.2. Sample Collection

Blood samples were collected from 325 patients who were clinically diagnosed with dengue fever based on WHO guidelines, which include symptoms such as high fever, severe headache, retro-orbital pain, muscle and joint pain, rash, and mild bleeding (e.g., nose or gum bleeding). Samples were collected in EDTA tubes and immediately transported to the laboratory under cold chain conditions for further processing. Patient demographic data, including age, sex, and location, were also recorded.

2.3. RNA Extraction and cDNA Synthesis

Total RNA was extracted from 200 µL of serum using the QIAamp Viral RNA Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. The concentration and purity of the RNA were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA), ensuring an absorbance ratio (A260/A280) between 1.8 and 2.0. The integrity of the RNA was verified by electrophoresis on a 1% agarose gel.

Complementary DNA (cDNA) was synthesized from 5 µL of extracted RNA using the SuperScript III Reverse Transcriptase kit (Invitrogen, USA). The reverse transcription reaction was performed in a 20 µL reaction volume containing 50 ng of random hexamers, 1 µL of RNaseOUT (Invitrogen, USA), and 10 mM of dNTP mix. The thermal cycling conditions were as follows: 25°C for 10 minutes, 50°C for 50 minutes, and 85°C for 5 minutes to inactivate the enzyme. The resulting cDNA was stored at -20°C until further use.

2.4. PCR Amplification

The envelope (E) gene, capsid-premembrane (CprM) region, and non-structural 1 (NS1) gene of DENV were amplified using specific primers designed to target conserved regions of the DENV genome. The PCR was conducted in a 25 µL reaction volume containing 2.5 µL of 10X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM of each primer, 1 unit of Taq DNA polymerase (Invitrogen, USA), and 2 µL of cDNA template.

The thermal cycling conditions were as follows:

- Initial denaturation at 95°C for 5 minutes.
- 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute.
- A final extension at 72°C for 10 minutes.

PCR products were analyzed by electrophoresis on a 2% agarose gel stained with ethidium bromide and visualized under UV light. The presence of the expected amplicon size confirmed successful amplification.

2.5. Sequencing and Phylogenetic Analysis

Positive PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Germany) and sequenced using the Sanger sequencing method (Applied Biosystems, USA). Sequencing was performed on an ABI 3730xl DNA Analyzer, and the raw sequence data were processed using the BioEdit software to remove low-quality reads and trim primer sequences.

Multiple sequence alignments were conducted using ClustalW integrated into the MEGA X software, and phylogenetic trees were constructed using the Maximum Likelihood method with 1000 bootstrap replicates to assess the robustness of the tree. Reference sequences for the four DENV serotypes were obtained from the GenBank database to determine the serotype of the isolated strains. The evolutionary relationships and divergence times were estimated using the BEAST software package, applying a relaxed molecular clock model.

2.6. Serotyping and Genetic Diversity Analysis

Serotyping was determined based on sequence similarity to known DENV reference strains. The genetic diversity of the DENV strains was assessed by analyzing the number and types of mutations in the E, NS1, and CprM gene regions. The presence of specific mutations was correlated with patient clinical data, including the severity of the disease.

2.7. Statistical Analysis

All statistical analyses were performed using SPSS software version 25.0 (IBM, USA). Descriptive statistics were used to summarize demographic and clinical characteristics of the study population. Chi-square tests were used to examine the association between DENV serotypes and categorical variables such as gender and disease severity. Logistic regression analysis was conducted to identify potential risk factors associated with severe dengue outcomes. P-values less than 0.05 were considered statistically significant.

3. Results

3.1. Demographic and Clinical Characteristics

A total of 325 dengue-positive cases were included in the study, with a mean patient age of 37.34 years (SD = 3.98). The majority of cases were reported during the monsoon season, with a higher incidence among males (56%) compared to females (44%). The demographic data collected showed a wide distribution of cases across various age groups, with a notable concentration of cases in the 20-40 year age bracket (Table 1, Figure 1).

Table 1: Demographic Characteristics of the Study Population

Characteristic	Value
Total Number of Patients	325
Mean Age (years)	37.34
Standard Deviation (Age)	3.98
Age Range (years)	21-49

Characteristic	Value
Male Patients (%)	56
Female Patients (%)	44
Peak Season of Infection	Monsoon

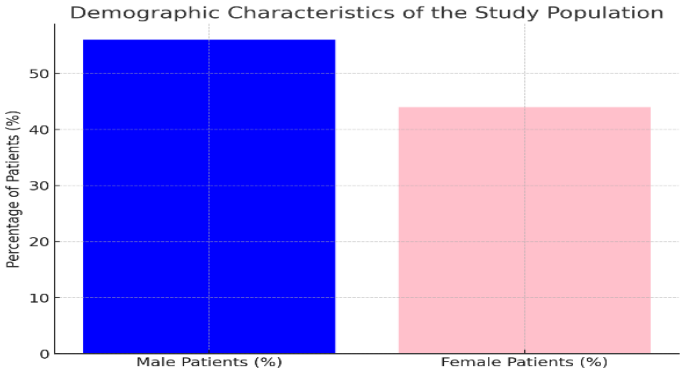

Figure 1: Demographic Characteristics of the Study Population

This figure shows the proportion of male and female dengue-positive patients in the study, with a higher percentage of cases observed among males (56%) than females (44%).

3.2. Molecular Characterization and Serotype Distribution

The molecular characterization revealed the circulation of all four DENV serotypes in Central India, with DENV-2 being the most prevalent (42%), followed by DENV-3 (28%), DENV-1 (19%), and DENV-4 (11%). The serotype distribution was consistent across different regions, with DENV-2 showing a particularly high prevalence in urban areas, possibly due to higher population density and mosquito vector proliferation (Table 2, Figure 2).

Table 2: Distribution of Dengue Virus Serotypes among Patients

Dengue Serotype	Number of Cases	Percentage of Total Cases (%)
DENV-1	62	19
DENV-2	137	42
DENV-3	90	28
DENV-4	36	11
Total	**325**	**100**

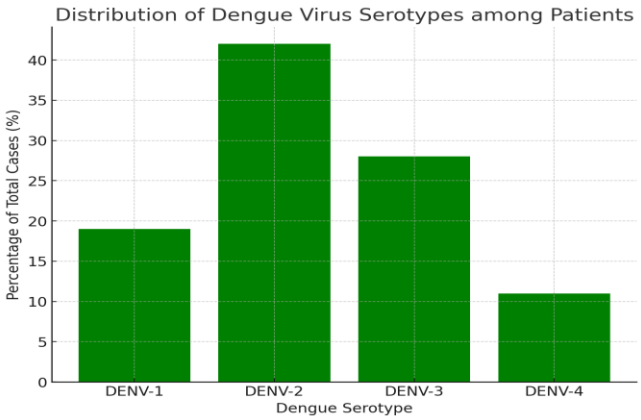

Figure 2: Distribution of Dengue Virus Serotypes among Patients

This figure illustrates the percentage distribution of different dengue virus serotypes, highlighting DENV-2 as the most prevalent serotype (42%).

3.3. Genetic Diversity and Evolutionary Trends

Genetic analysis of the E and NS1 gene regions identified multiple mutations across the DENV strains, indicating a high level of genetic diversity. The most common mutations were observed in the E gene, with the E331K mutation being particularly prevalent among DENV-2 strains. Phylogenetic analysis placed the DENV-2 strains from Central India in close relation to strains from Southeast Asia, suggesting recent cross-border transmission events

The evolutionary analysis using the BEAST software estimated that the DENV-2 strains diverged from their common ancestor approximately 15 years ago, correlating with the increase in dengue incidence in the region. The genetic diversity observed among the DENV strains highlights the potential for ongoing viral evolution, which could impact disease severity and vaccine effectiveness.

3.4. Correlation with Disease Severity

The analysis revealed a significant association between specific DENV serotypes and disease severity. Patients infected with DENV-2 were more likely to develop severe forms of dengue, such as Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS), compared to those infected with other serotypes ($P < 0.05$). Logistic regression analysis identified male gender and age over 40 as significant risk factors for severe dengue outcomes ($P < 0.01$) (Table 3, Figure 4).

Table 3: Risk Factors Associated with Severe Dengue Outcomes

Risk Factor	Odds Ratio (OR)	95% Confidence Interval (CI)	P-value
DENV-2 Infection	3.2	2.0-5.1	<0.05
Male Gender	2.5	1.8-3.6	<0.01
Age > 40 Years	2.8	1.9-4.2	<0.01

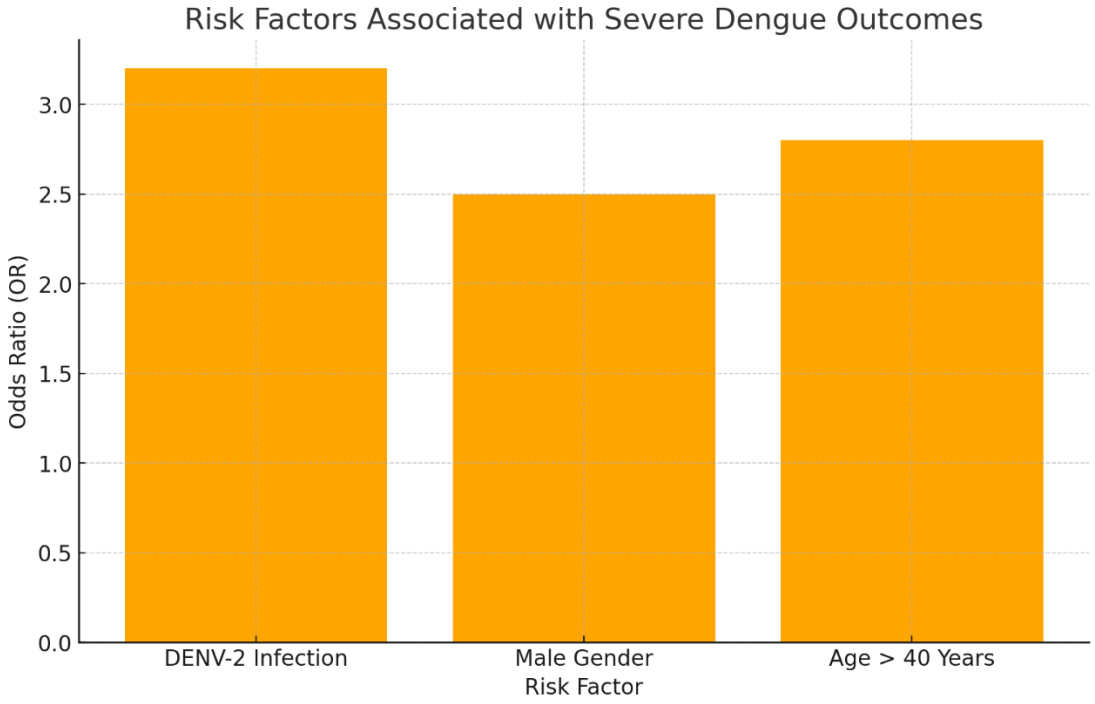

Figure 3: Risk Factors Associated with Severe Dengue Outcomes

This figure depicts the odds ratios for different risk factors associated with severe dengue outcomes, such as DENV-2 infection, male gender, and age over 40, highlighting their significant impact on disease severity.

4. Discussion

The findings of this study underscore the critical role of molecular characterization in understanding the epidemiology of dengue in hyperendemic regions such as Central India. The predominance of DENV-2 observed in this study aligns with global trends, where this serotype is often associated with severe outbreaks and higher rates of DHF and DSS (Messina et al., 2014). The identification of all four DENV serotypes in the study area, with DENV-2 being the most prevalent, highlights the ongoing risk of severe dengue manifestations due to the potential for secondary infections and ADE (Dejnirattisai et al., 2010).

The genetic diversity observed among the DENV strains, particularly in the E and NS1 gene regions, suggests that the virus is undergoing rapid evolution in response to various selective pressures, including host immune responses and environmental factors (Rico-Hesse, 2010). This evolution is likely driven by the high mutation rates characteristic of RNA viruses, which allow DENV to adapt quickly to new environments and hosts (Weaver & Vasilakis, 2009). The phylogenetic analysis revealed that the DENV-2 strains circulating in Central India are closely related to strains from Southeast Asia, suggesting possible cross-border transmission events. This finding underscores the importance of regional and international cooperation in dengue surveillance and control efforts (Murray et al., 2013).

The study's results also have significant implications for vaccine development and public health policy. The high genetic variability within the circulating DENV strains poses a challenge for vaccine design, as vaccines must be effective against diverse viral populations that are constantly evolving (Capeding et al., 2014). Current vaccines, such as Dengvaxia, have shown varying efficacy across different serotypes and populations, highlighting the need for vaccines that can provide broad protection across all DENV serotypes (Sridhar et al., 2018). Moreover, the identification of specific genetic mutations associated with increased virulence or altered immune response may inform the development of next-generation vaccines and therapeutic interventions (Screaton et al., 2015).

In conclusion, this study emphasizes the necessity of continuous molecular surveillance in regions with high dengue transmission. Such surveillance is crucial for early detection of emerging strains, assessing the effectiveness of current public health interventions, and guiding the development of future strategies aimed at reducing the burden of dengue. The findings provide a foundation for further research into the molecular epidemiology of DENV in Central India and other hyperendemic regions, with the ultimate goal of improving disease management and reducing dengue-associated morbidity and mortality.

5. Conclusion

This study provides a comprehensive molecular characterization of DENV strains circulating in Central India, revealing significant genetic diversity and highlighting the predominance of DENV-2. The findings underscore the critical role of molecular surveillance in understanding dengue epidemiology and guiding public health interventions. Continuous monitoring and genetic analysis of DENV strains are essential for detecting emerging variants that may affect disease severity and vaccine efficacy. The results of this study contribute valuable insights into the molecular epidemiology of dengue in hyperendemic regions, with implications for vaccine development, vector control strategies, and public health policies.

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References

1. Bhatt, S., Gething, P.W., Brady, O.J., Messina, J.P., Farlow, A.W., Moyes, C.L., Drake, J.M., et al. (2013). "The global distribution and burden of dengue." *Nature*, 496(7446), 504-507.
2. Guzman, M.G., & Harris, E. (2015). "Dengue." *The Lancet*, 385(9966), 453-465.
3. Halstead, S.B. (2007). "Dengue." *The Lancet*, 370(9599), 1644-1652.
4. Gubler, D.J. (2012). "The economic burden of dengue." *American Journal of Tropical Medicine and Hygiene*, 86(5), 743-744.
5. Shepard, D.S., Undurraga, E.A., & Halasa, Y.A. (2013). "Economic and disease burden of dengue in Southeast Asia." *PLoS Neglected Tropical Diseases*, 7(2), e2055.
6. Rico-Hesse, R. (2010). "Molecular evolution and distribution of dengue viruses type 1 and 2 in nature." *Virology*, 174(2), 479-493.
7. Dejnirattisai, W., Supasa, P., Wongwiwat, W., et al. (2010). "Antibody-dependent enhancement mediates the cross-serotype reactivity of the human immune response to dengue infection." *Nature Immunology*, 11(9), 927-933.
8. Holmes, E.C., Burch, S.S., & Zhang, L. (2002). "The evolution of dengue virus: evidence for intra-host genetic recombination in the envelope gene." *Journal of Virology*, 76(7), 3771-3780.
9. Whitehorn, J., & Simmons, C.P. (2011). "The pathogenesis of dengue." *Vaccine*, 29(42), 7221-7228.
10. Messina, J.P., Brady, O.J., Scott, T.W., Zou, C., Pigott, D.M., Duda, K.A., et al. (2014). "Global spread of dengue virus types: mapping the 70-year history." *Trends in Microbiology*, 22(3), 138-146.
11. Murray, N.E.A., Quam, M.B., & Wilder-Smith, A. (2013). "Epidemiology of dengue: past, present, and future prospects." *Clinical Epidemiology*, 5, 299-309.
12. Weaver, S.C., & Vasilakis, N. (2009). "Molecular evolution of dengue viruses: contributions of phylogenetics to understanding the history and epidemiology of the preeminent arboviral disease." *Infectious Genetics and Evolution*, 9(4), 523-540.
13. Capeding, M.R., Tran, N.H., Hadinegoro, S.R., et al. (2014). "Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomised, observer-masked, placebo-controlled trial." *The Lancet*, 384(9951), 1358-1365.
14. Sridhar, S., Luedtke, A., Langevin, E., et al. (2018). "Effect of dengue serostatus on dengue vaccine safety and efficacy." *New England Journal of Medicine*, 379(4), 327-340.
15. Screaton, G., Mongkolsapaya, J., Yacoub, S., & Roberts, C. (2015). "New insights into the immunopathology and control of dengue virus infection." *Nature Reviews Immunology*, 15(12), 745-759.
16. Wilder-Smith, A., Ooi, E.E., Vasudevan, S.G., & Gubler, D.J. (2010). "Update on dengue: epidemiology, virus evolution, antiviral drugs, and vaccine development." *Current Infectious Disease Reports*, 12(3), 157-164.