

Molecular Detection and Sero-Prevalence Study of Circulating Dengue Virus in Karnataka During 2024

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ABSTRACT

Dengue virus (DENV) is currently the most prevalent mosquito-borne viral pathogen. DENVs naturally exist as highly heterogeneous populations. Even though the descriptions on DENV diversity are plentiful, only a few studies have narrated the dynamics of intra-epidemic virus diversity at a fine scale. In the current study, was designed Prevalence Analysis of Circulating Dengue Virus Serotypes in Karnataka, India. We present a micro-scale genetic analysis of a monophyletic lineage of DENV-1 genotype III (epidemic lineage) detected from January to December 2024 in Karnataka, India by serological (NS1 antigen and IgM detection) and molecular techniques (Qualitative RT-PCR). Serum samples were collected from 825 patients with suspected dengue disease attending the OPD. Among all 475 samples were confirmed positive for dengue by ELISA. Of these cases, 131 showed NS1 antigen (27.58%), 219 showed IgM antibodies (46.11%), and 74 qualitative QRT-PCR (15.58) and 51 (10.74 %) serotyping RT-PCR. The prevalence rate of dengue fever was 17.9% during this study period. This study generates insights into the distribution of dengue serotypes and their association with disease severity. The DENV-2 was found as the dominant serotype in the outbreak 2024.

Keywords: : Dengue virus (DENV), Qualitative RT-PCR, NS1 antigen, IgM antibodies, ELISA.

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INTRODUCTION

Dengue is a significant arthropod-borne viral infection. It is a systemic, febrile disease caused by a mosquito-borne virus known as Dengue virus (DENV) (1), closely related to the etiologic agent of yellow fever (2). Dengue virus is one of the primary mosquito borne febrile infections affecting tropical and subtropical countries worldwide, and the disease is endemic in most countries globally (3).

The prevalence of dengue infection has increased drastically in recent decades. The disease is transmitted through the bite of female Aedes mosquito species (4). The enzootic cycle occurs among non-human primates in sylvatic habitats, where transmission is carried out by Aedes taylori and Aedes fucifer (5).

Dengue virus infection has spread to over 125 tropical and sub-tropical countries globally (6). The disease is endemic in most of these countries. According to the WHO, more than 2.5 billion people face the risk of infection annually, with 50 to 100 million cases occurring each year. Around 500,000 cases progress to dengue hemorrhagic fever (DHF), leading to approximately 24,000 deaths, predominantly in children (7). In many states across India, dengue transmission is classified as frequent or continuous.

The incidence rate of dengue case-fatality in India stands at 2.6% (8).

The Dengue virus is a single-stranded positive-sense RNA virus belonging to the genus "Flavivirus" and the family "Flaviviridae"(9). Its viral genome is approximately 11,000 base pairs long and contains a small 5' non-translated region (5' NTR) of about 100 base pairs, followed by a 10,000 base pair open reading frame (ORF)(10). This single ORF is translated into a polyprotein that is cleaved by both host and virus-derived proteases, resulting in the production of 3 structural proteins (Capsid (C), Membrane (M), and Envelope (E) and 7 non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5). The 5' NTR of the genome is capped with 7-methyl guanosine, and there is no polyadenylation in the 3' NTR (10, 11).

Depending on the genetic and antigenic properties, dengue virus infection is caused by four immunologically related serotypes: DENV 1-4 (10, 11). These serotypes exhibit significant antigenic differences and extensive genetic diversity within each serotype. Dengue infection in humans often presents with various clinical manifestations, ranging from mild febrile illness to severe hemorrhagic manifestations (14). Dengue fever typically manifests as an

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abrupt onset of febrile illness characterized by headache, severe pain in muscles and joints, and a rash lasting for 7 to 14 days (15). Severe dengue, previously known as DHF, is characterized by rapid capillary leakage, significant thrombocytopenia, and bleeding in the skin and gastrointestinal tract, among other hemorrhagic manifestations (16). In severe cases, there can be rapid loss of fluid into tissue spaces leading to hemo concentration and hypotension, which can be fatal. A small percentage of patients may develop severe vascular permeability, known as Dengue Shock Syndrome (DSS), which can be fatal if not promptly and appropriately treated (17). The occurrence of severe dengue correlates in most cases with the patient's immunological status, with Dengue shock syndrome primarily occurring in individuals experiencing their first dengue infection (16).

Confirmation of dengue virus infection can be achieved through various serological tests, including Enzyme-Linked Immunosorbent Assay (ELISA), virus detection via cell culture, identification of anti-dengue virus antibodies using hemagglutinin tests, and detection of viral nucleic acid through Reverse Transcriptase Polymerase Chain Reaction (RT-PCR), nested RTPCR, quantitative real- time RTPCR, and nucleic acid sequence – based amplification (NASBA) have been used for the detection of virus(21). Selecting genomic regions and identifying genetic variants of the dengue virus are critical for comparing sequences, understanding dispersion patterns, virulence, and recognizing strains responsible for epidemic outbreaks. Mutational changes in the DENV genome contribute to the emergence of new genotypes, which can affect virulence and disease severity (25, 26).

The current study aims to investigate and correlate sociodemographic and clinical parameters with infection severity. Specifically, it seeks to understand the

seroprevalence and molecular characteristics of dengue virus in the Southern region of Karnataka, where genetic information is currently limited.

2. Methodology:

2.1 Materials and Kits used in this study:

- QIAamp®ViralRNAMiniKit (QIAGEN)
- QIAasymphony® (QIAGEN)
- NucliSENS®easyMag® (bioMérieux)
- MagNAPure96System (Roche)
- m2000sp (Abbott)
- Maxwell®16lVDInstrument (Promega)
- Quant Studio 5®kPCR Molecular System (Thermo) Alternative nucleic acid extraction systems.
- TheRealStar®DengueTypeRT-PCRKit1.0containsaheterologousInternalControl (IC),which can either be used as a RT-PCR inhibition control or as a control of the sample preparation procedure (nucleic acid extraction) and as a RT-PCR inhibition control.

All reagents and samples used according manufacturer protocols by thawed completely, mixed (by pipetting or gentle vortexing).

2.2 Study population and sample collection:

The present study entitled “Prevalence and Genomic Analysis of Circulating Dengue Virus Serotypes in Karnataka, India” will be conducted in the Department of Microbiology, BMCRI Medical College, Bengaluru. For a period of 12 months i.e., from January to December 2024. A total of 825. Serum samples were collected from male and female patients of all ages with clinical features of dengue disease visiting Department of Microbiology, BMCRI Medical College, Hospital, Bengaluru were included in this study.

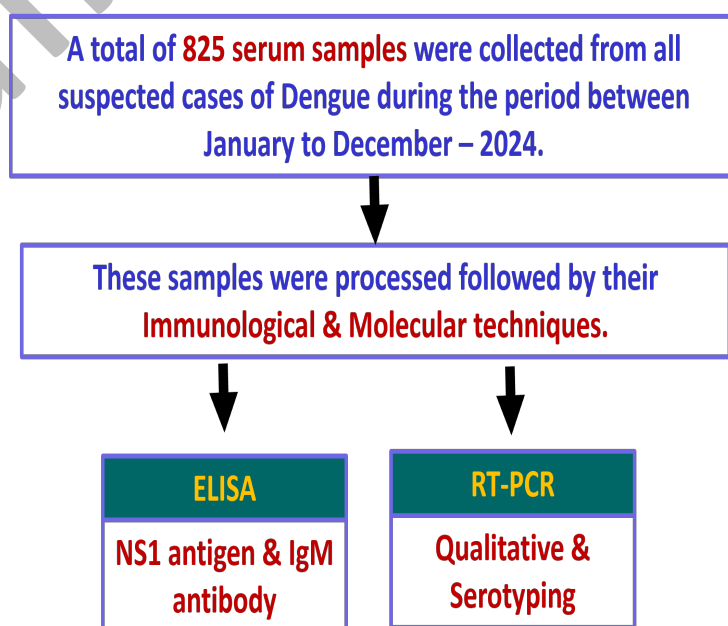


Figure 1: Flow Chart of study

2.3 Collection of the clinical samples and Ethical approval for this study:

The blood samples of about 5 ml were collected from symptomatic patients in a sterile clot activator vacutainer, along with clinical and demographic information from tertiary care hospitals located in various district of Karnataka, India. The blood samples used in this study were collected during dengue outbreak in Karnataka, between January -2024 to December 2024. This study was undertaken after obtaining the ethical clearance (Ref.No: BMCRI/EC/64/24) from the Ethics Committee of the Bangalore Medical College and Research Institute, Bengaluru. And the present study entitled “Prevalence Analysis of Circulating Dengue Virus Serotypes in Karnataka, India” was conducted in the Department of Microbiology, BMCRI Medical College, Bengaluru.

Before collecting the blood samples, informed consent was obtained from the participants. The samples were transported at 4 °C to the laboratory. Serum from the blood samples was separated and stored at - 80 °C until further analysis. All serum samples were screened seroprevalence studies by using Immunological tests ELISA by detecting DENV NS1 antigen and IgM antibody Kit (Standard Diagnostic Inc., Korea) according to the manual as well Molecular techniques like Dengue Qualitative REAL TIME PCR and Dengue Serotyping REAL TIME PCR.

2.4 Human serological testing:

2.4.1 Detection of dengue virus: Study on the molecular detection and Sero-prevalence of circulating dengue viruses typically involves two main components: molecular detection methods to identify the presence of the virus in biological samples, and sero-prevalence studies to determine the level of antibodies against the virus in a population (Mustafa et al., 2023).

ELISA Two different ELISA kits were used for the detection of NS1Ag and IgM in all the samples:

1. ELISA assay for NS1Ag: dengue NS1Ag was detected using an InBios dengue NS1Ag ELISA kit according to the manufacturer’s instructions.

2.5. ELISA assay for IgM: dengue IgM antibodies were detected using an IgM MAC ELISA kit by NIV Pune as per the instructions provided.

2.5 Dengue Qualitative RT-PCR & Dengue Serotyping RT-PCR:

Dengue Qualitative RT-PCR & Dengue Serotyping RT-PCR Detailed sociodemographic information of patients i.e., age, gender, clinical features and geographical area were documented.

RNA extraction: A QIAamp Viral RNA Mini kit (Qiagen) was used to extract viral RNA from serum samples of dengue-positive patients, as per the manufacturer’s instructions.

cDNA synthesis: As per the manufacturer’s instructions, reverse transcriptase PCR was used to synthesize cDNA from the extracted RNA using the Thermo Scientific RevertAid First Strand cDNA Synthesis Kit.

PCR assay for serotyping: PCR was performed in 350 dengue NS1Ag ELISA-positive samples using a primer set defined by Lanciotti et al. Briefly, we used 10 µl PCR master-mix 2× (Thermo Scientific), 1 µl forward primer (D1), 1 µl reverse primer (D2), 2 µl PCR-grade water and 6 µl cDNA; thermal-cycler conditions were 94 °C for 5 min, 94 °C for 30 s, 55 °C for 60 s and 72 °C for 120 s, 72 °C for 5 min and cooling at 4 °C. Serotypes DEN-1 to DEN-4 were detected by performing a second nested PCR using type-specific primers as defined by Lanciotti et al. (Table 1). The amplified product was then subjected to gel electrophoresis to analyze the result.

2.6 Statistical analysis: The collected data were checked, coded, and entered into graphed prism for analysis. Descriptive statistics were done and result was presented using text and frequency tables.

3. Results and Discussion:

The present study entitled “Prevalence and Genomic Analysis of Circulating Dengue Virus Serotypes in Karnataka, India” will be conducted in the Department of Microbiology, BMCRI Medical College, Bengaluru.

3.1 Evaluation of samples through Immunological and Molecular Techniques:

The samples collected were processed for further analysis. Of the total 825 patients of suspected dengue illness, a total of 475 patients were confirmed dengue illness either due to the presence of NS1 antigen or IgM antibodies by ELISA. Among these cases, 131 showed NS1 antigen (27.58%), 219 showed IgM antibody (46.11%), and 74 Qualitative RT-PCR (15.58%) and 51 Serotyping RT-PCR (10.74 %) (Table 3/Figure 3). The prevalence rate of dengue in this study period was 60.97%

Table 1: Number positive results for tested by Immunological and Molecular Techniques.

Test parameter	No. of positive cases	Percentage
NS1 Antigen	131	27.58
IgM Antibody	219	46.11
Qualitative RT-PCR	74	15.58
Serotyping RT-PCR	51	10.74
Total	475	

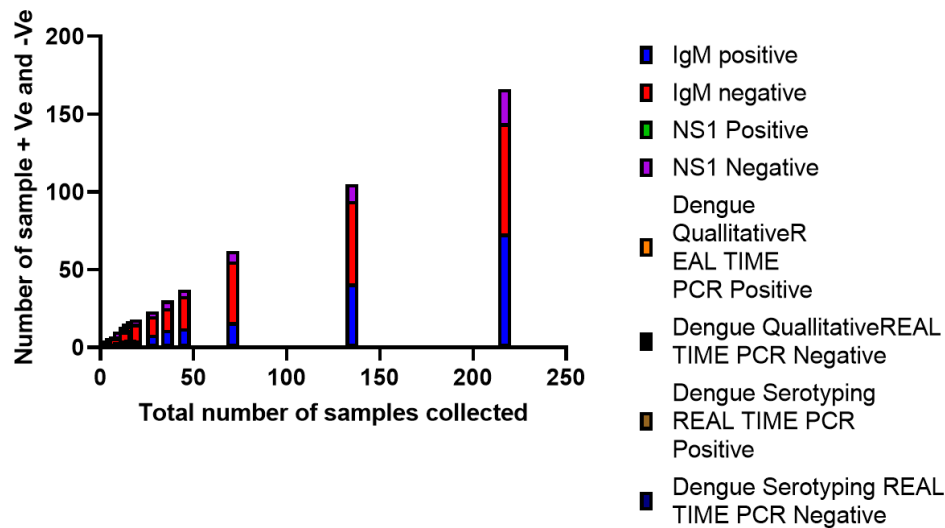


Figure 2: Number of test and its comparative analysis of +Ve and -Ve results

3.2 District wise distribution of dengue cases in Karnataka during year of 2024:

A total of 825 serum samples were collected from all suspected cases of Dengue during the period between January to December - 2024 from various districts of

Karnataka during January 2024 to December 2024. The detailed results analysis of its seroprevalence, Dengue Qualitative REAL TIME PCR and Dengue Serotyping REAL TIME PCR positive results were represented in table

Table 2: District wise distribution of dengue cases in Karnataka during year of 2024:

SL/NO	PLACE NAME	NUMBER OF SAMPLES COLLECTED	IgM		NS1		Dengue Qualitative REAL TIME PCR		Dengue Serotyping REAL TIME PCR	
			Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
1	Bengaluru Urban	270	85	185	59	211	36	49	25	34
2	Bengaluru Rural	162	46	116	33	129	19	27	13	20
3	Chikballapur	81	18	63	10	71	5	13	3	7
4	Kolar	53	13	40	9	44	4	9	4	5
5	Mandya	34	8	26	5	29	1	7	2	3
6	Mysuru	40	11	29	6	34	2	9	2	4
7	Tumkur	21	4	17	1	20	0	3	0	1
8	Shimoga	18	4	14	0	18	1	3	0	0
9	Hassan	21	4	17	0	21	1	3	0	0
10	Ballari	17	4	13	1	16	0	4	0	1
11	Hubballi	21	6	15	3	18	2	4	2	1
12	Udupi	14	3	11	0	14	0	3	0	0
13	Mangaluru	16	3	13	0	16	0	3	0	0
14	Chamarajanagar	24	5	19	2	22	2	3	0	2
15	kalburgi	9	2	7	1	8	0	2	0	1
16	Gadag	5	0	5	0	5	0	0	0	0
17	Belgum	8	1	7	0	8	0	1	0	0
18	Raichur	11	2	9	1	10	1	1	0	1
	TOTAL	825	219	606	131	694	74	144	51	80

Month wise distribution of dengue cases:

Table 3 : Monthwise distribution of Dengue cases

Month	No. of Dengue cases	Percentage
January	10	1.21
February	17	2.06
March	23	2.79
April	28	3.39
May	33	4.00
June	35	4.24
July	59	7.15
August	148	17.94
September	171	20.73
October	124	15.03
November	96	11.64
December	81	9.82

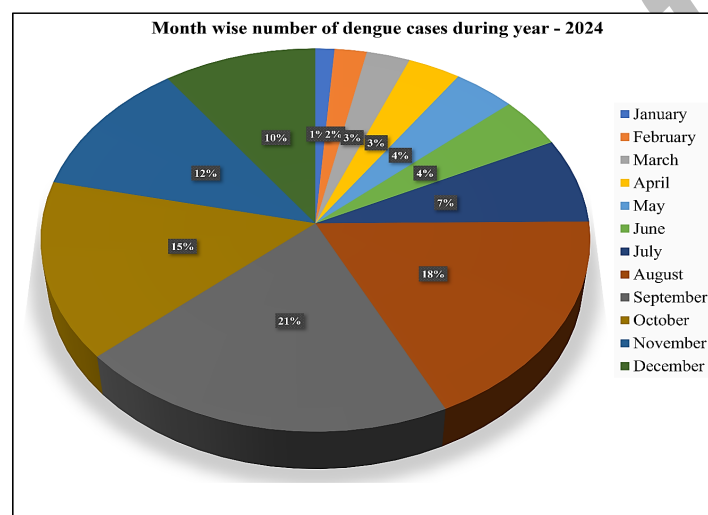


Figure 3: Month wise number of dengue cases during year – 2024

3.3 Age & gender wise distribution of dengue cases:

According to the present study, most of the patients 68.2% were in the age group of 20 to 60 years, totalling 562 patients. Patients above 60 years of age accounted for 8.97 % (74 patients) and below 20 years 22.91 % (189 patients) (Table 4/Figure 4). Out of 825 patients, most of the dengue

cases was observed among males 507 (61.45%) and incidence among females is 318 (38.55%). Maximum number of cases were in male was seen in the age group between 20 - 60 years i.e., 562 (68.12%).

Table 4: Age & gender wise distribution of dengue cases

Age Group	Number of Dengue Cases	Percentage
<20 years	189.00	22.91
20 – 60 years	562.00	68.12
>60 years	74.00	8.97
Gender		
Male	507.00	61.45
Female	318.00	38.55

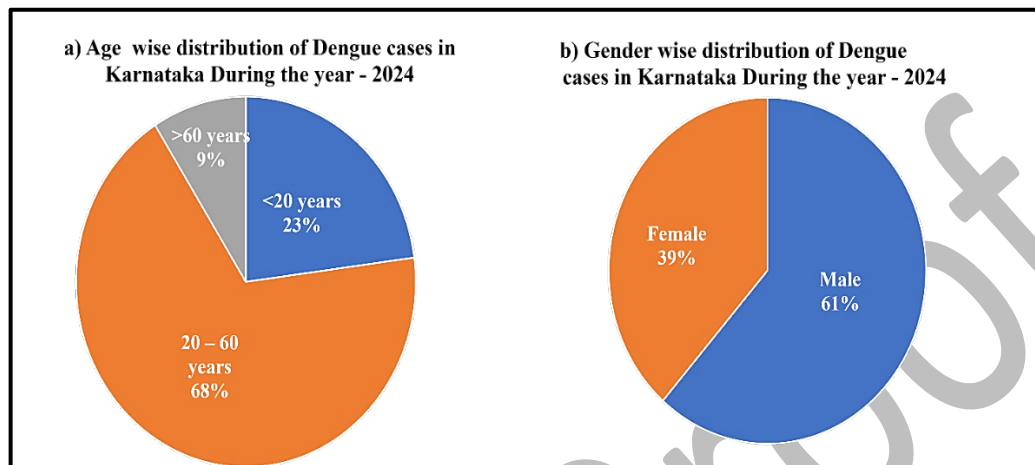


Figure 4: Age & gender wise distribution of Dengue cases in Karnataka during the year -2024.

3.4 RT-PCR confirmation of Dengue Virus:

A sample is positive for a serotype if a clear band of expected size is present and the corresponding positive control works. Negative for that serotype if no specific band is seen while the internal extraction control (host gene or spiked control) amplifies and the positive control amplified.

If no bands at all (including internal control)-invalid (possible extraction/PCR failure)-repeat extraction and PCR. If multiple serotype bands appear in the same sample-possible co-infection (report both serotypes; consider sequencing/confirmatory testing).

Table 5: Primer sequence and amplicon size for serotyping of DENV

Primer name	Sequence (5'→3')	Amplicon size (bp)	Location (with respect to ref. sequence)
nTS1	CTGGTTCGGT CTCAGTGATCCGGGGG	489	620-595
nTS2	AACGCCACA AGGGCCATGAACA	123	254-233
nTS3	TGCTGGTAAC ATCATCATGA GACAGAGCG	296	427-399
nDen4	CTCTGTTGTC TTAAACAGAGAGGGTC	395	527-502

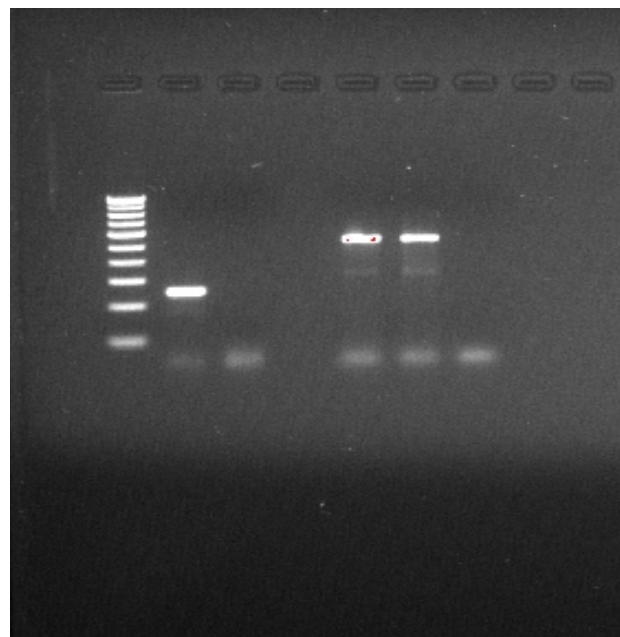


Figure 5: PCR confirmation of Dengue based on E gene

DISCUSSION

Dengue is a self-limiting, acute systemic viral infection caused by the rapidly spreading arthropod-borne Flavivirus. It is emerging as a major public health problem in India, affecting various parts of the country over the past two decades. India, along with six other Southeast Asian countries, is endemic for Dengue Fever (DF) and Dengue Hemorrhagic Fever (DHF), and it may soon play a significant role in the global burden of dengue infections. Unprecedented population growth and unplanned urbanization are the primary factors contributing to the spread of dengue virus infection in tropical developing countries. The identification of dengue cases is possible by distinct clinical features, but they can present with varied manifestations. The laboratory diagnosis of dengue infection is crucial, as the infection manifests with broad spectrum of clinical presentations and late diagnosis can lead to significant morbidity and mortality.

In this study, a total of 146 serum samples were collected from suspected cases of dengue during the study period and among them, 61 serum samples tested positive for dengue. Among these cases, 15(24.5%) NS1 antigen were detected, 24(39.3%) IgM antibody were detected, 12(19.6%) Qualitative RT-PCR & 10(16.3%) Serotyping RT-PCR were detected. A similar finding was seen in the study conducted by Bhardwaj et al⁽²⁷⁾, they have reported that the majority of cases presented in the first week of fever, 85.36% were positive NS1 antigen, followed by 20.8% IgM antibody positive. A similar study conducted by Rao et al.⁽²⁸⁾, showed alike results of NS1 antigen positive which was higher 71.4% compared to 18.3% IgM antibody positivity.

During this period of the study, a high rate of Dengue positivity was observed in June 35 cases (23.9%), followed by May 33 cases (22.6%), April 28 cases (19.1%), with the lowest rate seen in January 10 cases (6.8%). Similar findings were noted by Hemant Kumar et al.⁽³⁾ who reported the highest number of cases in June 2016. The seasonal trend of the disease is attributed to stagnant water accumulation during the pre-monsoon season, which provides a favourable environment for mosquito breeding. Additionally, favourable temperature and humidity during the monsoon season contribute to the increase in dengue cases. Rao et al.⁽²⁸⁾, also reported similar findings, with a higher number of cases occurring in June 2021 (43.8%). These findings underscore the importance of initiating preventive measures against dengue well before the monsoon season and maintaining them throughout the season.

According to the present study, the majority of patients 91 (58.2%) were aged between 10 and 60 years. Patients above 60 years of age accounted for 23.5% (17 patients). Kumar A et al⁽²⁹⁾, reported that the highest number of cases occurred in the age group of 15 to 44 years in their study.

Similarly, Shilpi Gupta et al⁽³⁰⁾, in their study in Uttar Pradesh reported a higher number of cases was observed in the age group 16- 50 years.

The highest number of cases among males was observed in the age group of 20 to 60 years, comprising 91 cases (58.2%). Ragini Singh et al⁽³¹⁾, also found a similar ratio of male to female dengue-infected patients in their study. Most studies indicate a higher prevalence of dengue infection among males, likely due to their greater involvement in outdoor activities during the daytime, increasing their exposure to mosquito bites.

CONCLUSIONS

The present study provides valuable epidemiological and molecular insights into the dynamics of dengue virus circulation in Karnataka during 2024. Out of 825 clinically suspected cases, 475 (57.57%) were serologically confirmed positive for dengue by ELISA, reflecting a substantial disease burden with an overall prevalence rate of 17.9% during the study period. The combined use of NS1 antigen detection, IgM antibody testing, and qualitative RT-PCR proved effective in capturing cases across different phases of infection, thereby improving diagnostic sensitivity and enabling a more accurate assessment of the true incidence of dengue in the region. Molecular serotyping revealed DENV-2 as the predominant circulating serotype during the 2024 outbreak, indicating a possible serotype shift or co-circulation pattern that warrants continued surveillance, given its established association with increased disease severity and risk of secondary infections. The detection of a monophyletic DENV-1 genotype III lineage further underscores the value of fine-scale genetic analysis in tracking intra-epidemic viral diversity, which is often overlooked in broader epidemiological surveys. These findings highlight the importance of sustained, serotype-specific molecular surveillance for the early detection of shifts in dominant DENV serotypes, which can inform clinical management, outbreak preparedness, and public health interventions. Continuous monitoring of circulating serotypes and genetic diversity will be crucial for anticipating changes in disease severity patterns and guiding vaccine and vector-control strategies in dengue-endemic regions.

Declaration of competing interest : The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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