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Circulating Dengue Virus Serotypes In Kolkata And Adjacent Districts During 2022-2024

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Abstract:

Background: *Dengue Viral fever is a major cause of concern and one of the leading causes of morbidity and mortality especially in tropical and subtropical countries. These viruses can lead to various illnesses ranging from mild to more severe conditions.*

Materials and methods*: A total of 1114 Dengue NS1 positive samples (482 hospitalized patients from IPGME&R-SSKM hospital, 328 and 304 patients from South 24 pgs and Howrah district hospitals respectively) were collected from October 2022 to October 2024 for Dengue serotyping study.*

Results: *Out of total 887 Dengue serotype detectable samples, 32 tested positives for DENV-1 (3.60%), 545 for DENV-2 (61.44%), 271 for DENV-3 (30.55%) and 4.39% (N=39) for DENV-4. As per our study, DENV-2 was major circulating stain from 2022 onwards up to 2024 followed by DENV-3 (30.55 %). DENV-1 (3.06%) and DENV-4 (4.39%) were mainly seen in the year 2024.We also observed that from June onwards in every year Dengue infection gradually increases and high peak was seen in the month of October and November in three consecutive years.*

Conclusion: *For Dengue serotype detection, Real Time PCR is the gold standard method with high sensitivity and specificity. We can detect multiple serotypes in the single tube Multiplex Real Time PCR method in a very short period of time. Policymakers, physicians, and other healthcare professionals will benefit from this study to regulate vector-borne virus outbreaks and employ molecular techniques for early detection.*

Keywords: *Dengue serotype, Dengue infection, Real Time PCR, molecular diagnosis*

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I. Introduction

Dengue fever is an acute systemic viral disease caused by the dengue virus, which is primarily transmitted to humans through the bites of infected *Aedes aegypti* mosquitoes (1). Dengue virus is an arthropod-borne, enveloped, single-stranded RNA virus from the Flaviviridae family (2). Dengue fever is a public health concern with significant cause of morbidity and mortality, particularly in tropical and subtropical regions, including Southeast Asia, the Caribbean, Central and South America, and parts of Africa (1,3). It is characterized by high fever, severe headache, body ache, pain behind the eyes, joint and muscle pain, pain in abdomen, rash, and in severe cases, bleeding shock syndrome or organ failure (4). India is indeed one of the global epicentres of dengue, with a significant burden of clinical infections. According to global data, an estimated 33 million clinically apparent dengue cases occur in India each year (5). This makes India responsible for about one-third of the total global dengue burden (5,6). The impact of dengue in India is not only felt in terms of the number of cases but also in terms of the economic burden on healthcare systems and communities. While many cases are mild and self-limiting, but severe forms of dengue, such as dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS), can be fatal without timely medical intervention (4).

Dengue virus is classified into four antigenically different serotypes: DENV-1, DENV -2, DENV -3, and DENV -4. In 2013, the fifth serotype (DENV-5) was identified in Malaysia though this type is not common (7). DENV-1 and DENV-2 serotypes were first isolated from U.S. soldiers during World War II whereas DENV-3 and DENV-4 serotypes were isolated later in 1954 from patients with DHF in the Philippines and Thailand (8). In India, all four serotypes were first documented in Vellore city, Tamil Nadu in the year 1968 (2). 1993 and 1996 outbreaks in India were caused by Dengue virus-2, which was the most common serotype between the 1970s to 2000 (8). Delhi's 2003 and 2006 outbreaks were primarily caused by the Dengue virus-3 strain (2). A study showed that from 2008 to 2010, the common circulating dengue serotypes in and around Kolkata were DENV-1, DENV-2 and DENV-4 (8). However, from 2011 onwards, the DENV-3 serotype began to rise in prevalence, leading to a significant dengue outbreak in 2012 (8). DENV-4 and DENV-3 were detected in the year 2020 and 2021

respectively in West Bengal (3). Dengue serotyping identification in various region of a country is very essential for epidemiological knowledge and subsequent vaccine development.

The aim of the study to present an overview the common circulating Dengue serotype in Kolkata and adjacent districts during 2022 to 2024. We will also detect epidemiological findings and seasonal viral surge throughout the year and predominant serotype year wise in this region.

II. Materials And Methods

Study duration: October 2022 to October 2024.

Study Design: Hospital based observational study.

Study Location: Institute of Post Graduate Medical Education and Research (IPGME&R),Kolkata.Dengue stereotyping panel by Real Time PCR were performed at Virus Research and Diagnostic Laboratory (VRDL), unit of Microbiology department of the Institute of Post- Graduate Medical Education and Research and Seth Sukhlal Karnani Memorial Hospital (IPGME&R and SSKM Hospital).

Sampling size: A total of 1114 Dengue NS1 positive samples (482 hospitalized patients from IPGME&R and SSKM hospital, 328 and 304 patients from adjacent South 24 pgs and Howrah district hospitals respectively) for Dengue serotyping study. Patients age ranged from 1 month old baby to80 years old senior adult and 610patients were females and 504individuals were males.

Ethical considerations: This study protocol was approved by the Institutional ethical committee of Institute of Post Graduate Medical Education and Research (IPGME&R), Kolkata

Methodology

RNA Extraction:

Underbioseftylevel-IIB2cabinet, all the serum samples were sorted out serially. RNA extraction process was performed by MagMAX Viral/Pathogen II (MVP II) nucleic acid isolation kit using KingFisher automated extraction system (Thermo Fisher Scientific) according to manufacturer's protocol (9). Briefly, Kingfisher instruments automate extraction of RNA, using magnetic beads that capture targeted nucleic acid. Beads bind the nucleic acid more efficiently than glass-fiber filters, resulting in higher and more consistent yields. Using a simple workflow including binding, washing, and elution. KingFisher instruments can automate the extraction of any analyte of interest. Once captured, these nucleic acids can then be eluted in elution buffer for use in downstream applications (9).

Detection of dengue serotypes by RT-PCR:

Isolated RNA was then subjected to RT-PCR using Himedia PCR Dengue Stereotyping PCR Kit (10). This technique is used to amplify a targeted DNA sequence by use of hydrolysis probes that are short oligonucleotides that have a fluorescent receptor dye attached to the 5' end and a quencher dye to the 3' end. Hi PCR Dengue Stereotyping Probe PCR Kit is designed to detect the polyprotein gene of Dengue serotypes 1,2,3 and 4 in FAM, JOE, CY5, Texas Red Channels Respectively with Internal control in Cy5.5 channel in a single tube reaction. 20 microlitre reaction was standardised as follows: 5 µL of cDNA with each PCR mixture containing 5 µL of RT Buffer, 2.5 µL of 10 X solution, 1µL of M- Mulv Reverse Transcriptase, 4 µL of Den-1-4 Primer Probe Mix, 1 µL of Internal Control DNA, 5 µL of nuclease-free water.The RT-PCR reaction is thermally cycled condition as follows: 15 minutes of RT at 50 °C, 2 minutes 30 seconds of initial denaturation at 95 °C, denaturation at 95 °C for 15 s, annealing and extension at 61 °C for 01 min, Ultimately, a real-time PCR machine was utilized to set up the reaction after adding 5 µL of RNA taken from the clinical sample to the 20 µL RT-qPCR master mix.In reaction A, the fluorescent labels utilize in the probes were FAM for DENV-1, HEX for DENV-2, CY-5 for DENV-3, TEXAS RED for DENV-4; CY-5 for Internal control.Every fluorescent dye's cycle threshold (Ct) value was evaluated separately. A positive sample was one with a Ct value of less than 37 (10).

III. Results

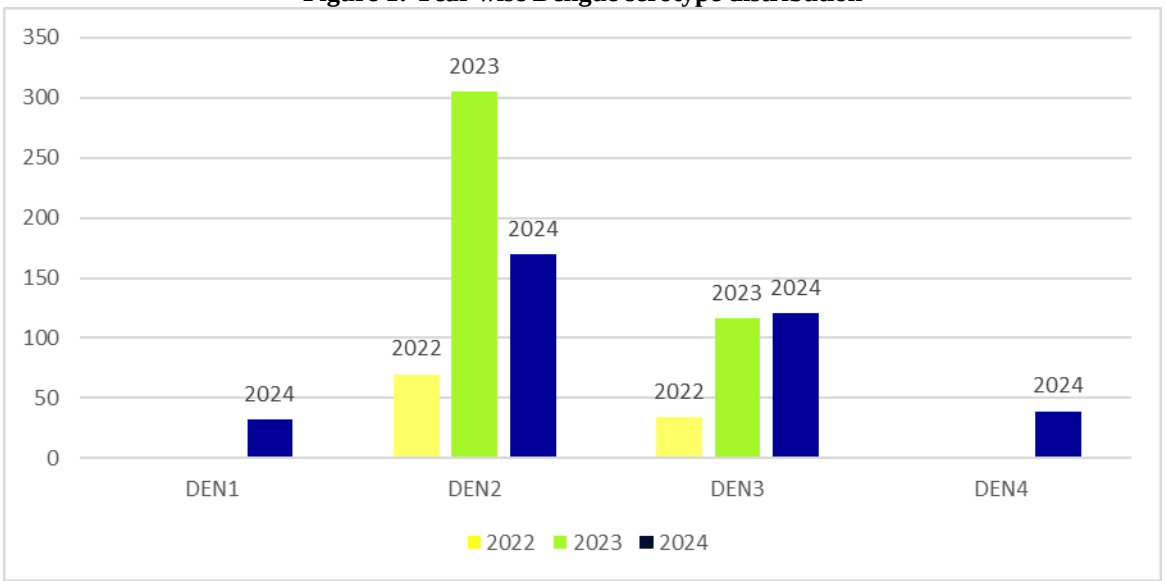
A total of 1114 Dengue NS1 positive samples were collected from October 2022 to October 2024. Out of the total number of cases, 54.45% (N=610) were females and 45.24% (N=504) were males. Their age ranged from 1 month old baby to 80 years old adult. Of the total, 764 (68.58%) patients were from urban or semi urban area and rest 350 (32.41%) were from the rural area (Table-1). As per results, age range from >20 years up to 40 years were mostly infected than other age groups. Age wise distribution of dengue serotype ware presented in table-1.

Table 1: Demographic profile and Dengue serotype distribution age group wise

Characteristics	Total Number of Dengue NS1 positive samples (N=1114)	Total Number of Dengue Serotype detected (N=887)	DENV-1 (N=32, 3.06%, out of 887)	DENV-2 (N= 545, 61.44%, out of 887)	DENV-3 (N=271, 30.55% out of 887)	DENV-4 (N=39, 4.39%, out of 887)
Demographic Profile						
Urban or Semi urban Area	764 (68.58%)	581 (65.50%)	30 (5.13%)	332 (57.14%)	180 (30.98%)	39 (6.71%)
Rural Area	350 (32.41%)	306 (34.49)	10 (3.26%)	150 (49.01)	136 (44.44%)	10 (3.26%)
Gender						
Female	610 (54.45%)	521 (59%)	20 (3.83%)	312 (59.88%)	160 (30.71%)	29 (5.56%)
Male	504 (45.24%)	366 (41%)	12 (3.27%)	233 (63.66%)	111 (30.32%)	10 (2.73%)
Age Groups (Years)						
>0-10		35 (3.94%)	04 (11.42%)	19 (54.25%)	10 (28.57%)	02 (5.71%)
>11- 20		91 (10.93%)	04 (4.39%)	71 (78.02%)	14 (15.38)	02 (2.19 %)
>21-30		297 (33.48 %)	06 (2.02%)	154 (51.85%)	133(44.78%)	04 (1.34%)
>31-40		249 (28.07 %)	05 (02%)	162 (65.06%)	72 (28.91%)	10 (4.01%)
>41-50		98 (11.04 %)	06 (6.12%)	78 (79.59)	10 (10.20)	04 (4.08)
>51-60		71 (8.04 %)	03 (4.22%)	40 (6.72) (56.33%)	19 (26.76%)	09 (12.67%)
>61-70		22 (2.48 %)	02 (9.09%)	10 (45.45%)	06 (27.27%)	04 (18.18%)
>71-80		24 (2.70 %)	02 (8.33%)	11 (45.83%)	07 (29.16%)	04 (16.66%)

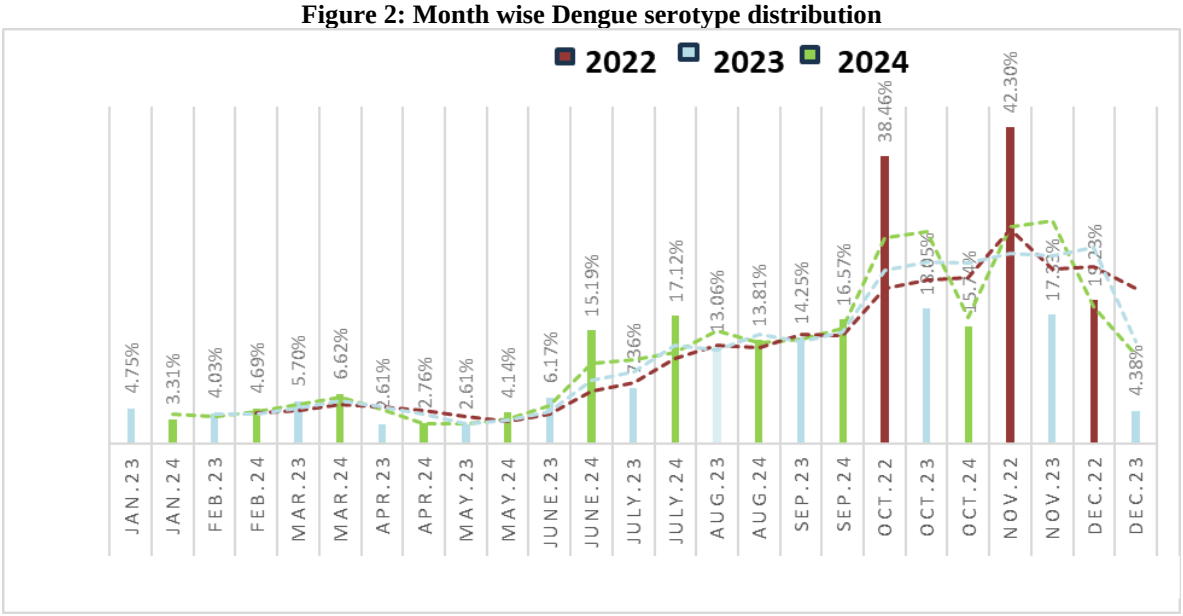
Viral RNA extraction was performed of 1114 NS1 positive samples in which we detected Dengue serotyping of 887 samples by Real Time PCR. Out of total 887 Dengue serotype detectable samples, 32 tested positives for DENV-1 (3.60%), 545 for DENV-2 (61.44%), 271 for DENV-3 (30.55%) and 4.39% (N=39) for DENV-4. As per our study, DENV-2 was major circulating stain from 2022 onwards up to 2024 followed by DENV-3 (30.55 %). DENV-1 (3.06%) and DENV-4(4.39%) were mainly seen in the year 2024 (Figure. 1).

Figure 1: Year wise Dengue serotype distribution



We also observed that from June onwards in every year Dengue infection gradually increases and high peak was seen in the month of October and November in three consecutive years (Figure-2).

Figure 2: Month wise Dengue serotype distribution



IV. Discussion

We carried out a tertiary level hospital-based study in Kolkata and adjacent districts to find out the circulating Dengue serotype with a sample size of 1114 Dengue NS1 positive samples in the period from October, 2022 to October, 2024. Within the infected cases, gender wise distribution shows that females to be more vulnerable to infection compared to males. The infected female population was found high due to they are mainly involved in household activity than males. The majority of positive patients comes from urban or semi urban area in comparison to rural settings. Urban area has a higher population density with a public transit system, greater global connection, poor sanitation and vector control surveillance all of which contribute to the spread of dengue virus infection faster.

We detected serotyping of only 887 samples by Real Time PCR. As per our Study, DENV-2 (61.44%) was major circulating stain followed by DENV-3 (30.55%) in three consecutive year (2022 to 2024). A small number, 3.06% and 4.39% of DENV-1 and DENV-4 respectively were seen in 2024 along with DENV -2 (46.96%) and DENV-3 (33.42%). Same pattern of circulating Dengue serotypes was found in Kolkata and adjacent two districts. A study conducted in northern part of West Bengal in 2022, found that DENV-3 was major circulating serotype (11). Dengue infected Patients were categorized in different age group to check age wise positivity. Age >20 years up to 40 years were found major infected population. People in their middle years frequently spend more time outside because of their jobs, social lives, or travel that expose them to mosquito vectors more frequently. This makes it more likely that the virus may infect people in endemic locations. As per our observation, post monsoon months onwards Dengue viral infection gradually increases. In the month of October and November highest peak was seen in three consecutive years due to delayed monsoon season. Environmental and ecological changes due to global warming make the post-monsoon season a vital time for the increase in dengue virus transmission (12). The warm and humid conditions that typically follow the monsoon create an optimal environment for the growth of mosquito populations. Aedes mosquitoes thrive in temperatures between 25°C to 30°C (77°F to 86°F), which is common after the monsoon rains (13). During the monsoon, heavy rains create numerous puddles, waterlogged areas at construction sites and stagnant pools, all of which are ideal breeding grounds for Aedes mosquitoes, the primary vectors of the dengue virus. This leads to higher chances of human-mosquito contact and subsequent virus transmission.

V. Conclusion

Nowadays Dengue Viral fever is one of the leading and most common infections effecting the all-age groups. These viruses can lead to various illnesses ranging from mild to more severe conditions. Due to the intense climatic change, the Dengue viruses are present roughly all throughout the year but the major peak was noticed post monsoon month (October, November). For Dengue serotype detection, Real Time PCR is the gold standard method with high sensitivity and specificity. We can detect multiple serotypes in the single tube Multiplex Real Time PCR in a very short period of time which will be of great help to all the clinicians to give the treatment accordingly. Region wise molecular epidemiological study on Dengue serotyping will give the information to the policy makers, clinicians and healthcare workers to take necessary steps to control the vector borne viral outbreaks in future.

Conflict of Interest:
The authors declare they have no conflict of interest.

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