Epidemiology of Dengue and Chikungunya Infection in Bangalore, Karnataka – 2024

Abstract

Background: Dengue viral infection is emerging as a serious public health problem in Karnataka. An enhanced testing facility can generate information on the epidemiology of the dengue disease, which is mandatory for planning and development of relevant control & preventive action against Dengue.

Materials and methods: A prospective study was carry out between January 2024 to July 2024, by testing suspected Dengue patients attending Private Medical College & Hospital in Bangalore, Karnataka, India) to define the status of Dengue burden, the natural history of this disease in terms of clinical presentation and outcome of the infections in hospitalized Dengue patients. The sample received from suspected patients were analyzed for Dengue specific RTPCR using Fast Track Diagnostic kit (FTD Tropical Fever Kit) and Dengue subtyping were performed with type specific primer using High resolution melt cure analysis and gel electrophoresis. The clinical case described by World Health Organization (WHO) was adopted to classify the Dengue cases.

Results: The total number of samples tested during the period was 195, out of which 24 (12.3%) were positive for Dengue and 19 (9.7 %) for Chikungunya. By clinical evaluation, Dengue fever (DF) was diagnosed in 15 patients, 4 had hemorrhagic manifestations and 1 patients had progressed to DSS. Though (DSS+DHF) was present in 20 patients, all of them recovered well.

Conclusion: In developing countries like India, building of diagnostic laboratory with advanced facility for diagnosis and combat-mode ready preparedness for the management of Dengue cases in emergency situation may reduce Dengue-related mortality.

Key words: Dengue, Chikungunya, Dengue shock syndrome, Dengue Haemorrhagic fever,

Introduction

Chikungunya (Alphaviruses) and Dengue viruses (Flaviviruses), both are mosquito-borne diseases and now-a-days it turns a serious epidemic in the past few decades, most commonly in the tropical region like India. CHIKV was not recognized until the early 1950s in East Africa and it was first isolated in 1952. (1) In late 1800s from 1880 to 1955, first Dengue

Fever was in Australia. In history of the ancient disease in Chin Dynasty of 265-420 AD had similar symptoms with dengue. (2&8) According to Centers for Disease Control and Prevention (CDC) CHIKV viral disease and outbreaks in more over 100 countries in Africa, USA, Asia, UK and Indian and Pacific oceans regions. As of March 5, 2024 had evidence of CHIKV transmission among humans within past 5 years, Asian countries like Cambodia, China, India, Indonesia, Laos, Malaysia, Maldives, Myanmar, Pakistan, Philippines, Taiwan, Thailand, Timor leste and Vietnam are affected by CHIKV infection. (3)

India, an endemic area for Chikungunya, Dengue and Malaria had seasonal infection and most importantly Chikungunya and Dengue virus are transmitted by same vectors and show similar clinical symptoms. There is no specific anti-viral drug drugs or regimen available for Chikungunya, many of therapeutic options are under investigation. CHIKV infected patients are requested to take huge amount of water or any fluids to prevent dehydration. Usually, paracetamol or acetaminophen were used to reduce fever and pain. (4) According to CDC, vaccination against Dengue is only a part of integrated strategy to control the infection to avoid severe health problems to mild issues. likewise, TAK-003 does not protect dengue infection in all the cases. (5) In Dengue, there are four different serotypes namely DEN 1,2,3 and 4, but in October 2013 – Sarawak a state of Malaysia has been isolated and analysed the genomic sequence of the DEN 5 a fifth serotype. Non-structural protein 1 (NS1), DEN genome variation, antibody-dependent enhancement (ACE) and memory cross reactive T cells are the attributes of Dengue pathogenesis. (6)

CHIKV RNA viruses were recognized by Specific pattern recognition receptors (PRRs) and presence of infection. Retinoic acid Inducible gene (RIG) - I – like receptors (RLRs) which includes RIG-I, Anti-melanoma differentiation-associated gene 5 (MDA-5) will sense the RNA virus in cytoplasm. Toll-like receptors (TLRs) like TLR-3, TLR-7 & TLR-8 will sense the virus in endosomal region. After the viral identification RLRs, TLRs, Interferon regulatory factor 3 (IRF-3 & 7) and nuclear factor kappa-B (NF- κB) were activated. (7&10)

In Karnataka, 16986 cases and 13 deaths reported in 2019; 3823 cases in 2020; 7393 cases and 7 deaths in 2021; 9889 cases and 9 deaths in 2022; 19300 cases and 11 deaths in 2023 and in 2024 till February 2503 cases with nil mortality reported by NCVBDC. (9). The present study reports the Current status of Chikungunya and Dengue infections and Dengue subtype circulation in Karnataka, India.

Methods

Microbiological Laboratories, Bangalore, located in the state of Karnataka, India. Blood samples were collected from the surrounding hospitals, patients suspected with febrile illness, Dengue hemorrhagic fever with clinical symptoms of headache, joint pain, arthralgia, rashes myalgia and leucopenia. Samples were collected after the clinical symptoms in 3 – 7 days, and processed within a day. Stored specimens are not used for processing. A minimum of 2 ml blood sample was collected in Ethylenediaminetetraacetic acid (EDTA) collection tubes for plasma. Suspected blood samples were tested by using Real-Time Polymerase Chain Reaction (RT-PCR) with Tropical fever core kits (Fast track Diagnostics) to detect the Chikungunya virus & Dengue virus; later, four distinct serotypes were analysed using High resolution melt curve analysis (HRMA) technology.

Extraction of RNA

EDTA blood samples were subjected to DNA extraction using QIA amp viral RNA kit (Qiagen, Germany) 200µl vortexed blood samples were mixed with 560µl of a viral lysis buffer (AVL) kept 10 minutes incubation at room temperature and the extraction followed with the manufactures protocol. Known positive and negative samples taken from the Chikungunya virus and Dengue virus (DEN I, II, III & IV) infected individuals and un-infected individuals. Extracted RNA elution were stored at -20 °C for later use.

Target amplification

Chikungunya virus and Dengue virus are detected using Tropical fever core kits (Fast track Diagnostics) in Magnetic induction technology (MIC) RT-PCR. A total volume of 25µl of master mix, includes 10µl of extracted RNA, 2µl of primer-probe (TF1 &TF2) and other components kit (FTD). Thermocycler initiated with reverse transcription at 42°C for 15 minutes; one long denaturation at 94°C for 3 minutes and the following steps includes 40 cycle repeats of denaturation at 94°C for 8 seconds; 60°C for 34 seconds extension. After amplification, run was analysed by MIC RT-PCR software. Tropical Fever core tube 1 containing Dengue virus, *Rickettsia* spp, West Nile virus and *Salmonella* spp; Tropical Fever core tube 2 containing *Plasmodium* spp, Chikungunya virus, *Leptospira* spp. Chikungunya virus and Dengue virus were analysed and reported. Later, Dengue serotypes were detected by RT-PCR HRMA technology.

Serotype Detection

Dengue positive elutes were separated for the serotype identification, DEN virus had D1, D2, D3 and D4 were amplified in MIC RT-PCR (Biomolecular system, Australia) using HRMA technology. 8µl of dengue positive RNA elute, 0.5µl of reverse transcriptase enzyme, 2µl of primer for D1, D2, D3 & D4 and other components of Type-it HRM kit (Qiagen). Amplification initiated with reverse transcription at 42°C for 20 minutes; one cycle denaturation and activation of Taq polymerase at 95°C for 5 minutes and 40 cycles at 95°C for 10 seconds, 55°C for 30 seconds and 72°C for 15 seconds; and High-resolution melt initiated at 70°C to 93°C raising 0.1°C with 2 seconds hold for each step. After the amplification run was analysed in MIC RT-PCR software. Based on their sero-specific melt Dengue virus serotypes determined. After melt cure analysis, a 5 µl amplicon of each product was analyzed by agarose gel electrophoresis, and the serotype was idendified by the amplicon size as indicated below [Figure 1]. For each RT-PCR run, a positive control (PC) and a negative control (NC) was included. An aliquot of 10 µl of the RT-PCR products was analyzed on 1.5% agarose gel electrophoresis, stained with ethidium bromide and visualized under UV-light. The size of the RT-PCR products from the amplification of DEN-1 -208bp, DEN-2 -119bp, DEN-3 -288bp and DEN-4 -260bp [10] The PCR gel was stained with ethidium bromide and run at about 100 volts. The gel was viewed under Alpha Imager (Alpha InnotechSan Diego, California, USA) and the resulting bands were captured with a polaroid camera.

Results

Overall enrolment of 547 cases were suspected for Chikungunya and Dengue infection. out of this, 55 (10.1%) individuals were detected positive for Chikungunya; 32 (5.8%) individuals were detected positive for Dengue infection and 1 (0.18%) person infected by both Chikungunya virus and Dengue virus [Table 1]. Of the 547 cases (202 males, 150 females), 34 (61.8%) males and 21 (38.2%) females were detected positive for Chikungunya virus; 16 (50%) males and 16 (50%) females were detected positive for Dengue virus.

Of the 547 cases, 11 (20%) were less than 10 years old for Chikungunya virus and 45 (80%) were more than 10 years old; In case of Dengue virus, 6 (18.75%) children were positive and 26 (81.25%) were more than 18 years old. The samples collected among the age group of 0 – 82 years old and the mean age was 41. Chikungunya infection significantly high in pediatric age group (0 – 10 years old); Dengue infection scattered among all age groups especially had

high positivity in middle age people (21 – 30 years old). Dengue infection in infants (4) were only males; 3 cases – (11-20 old); 9 cases – (21-30 old); 6 cases – (31-40 old); 10 cases – (41-82 old) and Chikungunya infection in 11 cases – (0-10 old); 1 case (11-20 old); 6 case (21-30 old), 10 cases (31-40 old), 27 cases (41-82) [Table 2]. Analysis of Dengue serotype shows 8 cases were positive for DEN 1,3 and 4 [Figure 1]; 3 cases were positive for DEN 1 and 4 [Figure 2]; 3 cases were positive for DEN 1 and 3; 8 cases positive for DEN 1; 7 cases positive for DEN 3; 3 cases positive for DEN 4; DEN 2 were remains not detected in this study [Table 1].

Clinical symptoms of among both Chikungunya and Dengue infected (87 cases) individuals, 76 cases had high fever with body pain, 64 cases had haemorrhagic manifestation and 49 cases were severe infection with vomiting sensation. During the study period mortality rate of Chikungunya and Dengue was nil due to the timely diagnosis and good clinical practices. In this study, Chikungunya 24 cases positive with 26 high level, 25 medium level and 4 low level positives; Dengue 32 cases positive with 20 high level; 16 medium level positives and 1 low level were observed. January to December 2024 – 15 cases of Chikungunya and 10 cases of Dengue were detected in July 2024. Increased number of Positive cases were observed in July 2024. Chikungunya infection in January – 1 case, March – 1 case, June – 7 cases, July – 15 cases, August – 11 cases, September – 7 cases, October – 6 cases, November – 4 cases and December – 3 cases; Dengue infection in March – 5 cases, April – 1 case, May – 2 cases, June – 1 case and July – 10 cases, August – 4 cases, September – 7 cases, October – 1 case and November – case [Table 3]. All the Chikungunya and Dengue infected persons were admitted and others were treated as outpatients.

Discussion

In this study, increased positive cases of Chikungunya and Dengue were observed in July 2024 and no. cases were observed in February 2024. Analysis of Dengue serotype shows high possibility of DEN 1 infection alone or combined, DEN 2 were remains not detected among the total enrolment. Infants and Middle age people had high possibility of Dengue infection; Children under pediatric (Six years old to ten years old) are highly infected with Chikungunya. Twenty individuals were reported high level positive for Chikungunya with severe fever. According to World Health Organization increased cyclic epidemics are occurring in India, Bangladesh and Maldives - dry and wet climatic condition favours high

risk of spreading multiple serotype viruses in similar areas. In India case-fatality in urban areas were up-to 3-5% rest of the other countries had 1%. (11)

Survey of National Centre for Vector Borne Disease Control, increased incidence of dengue in past few years in 2018, a total of 124,493 cases; 205,243 in 2019; 44,585 in 2020; 193,752 in 2021 and 233,251 in 2022. In every year July – November had an upsurge in dengue infection, *Aedes aegypti* is a main vector in urban areas; *Aedes albopictus* also incriminated in many states. Temperature, humidity influence and grow of female mosquitoes, best at 16°-30°C and over 60-80%. (12) WHO, resurgence of Chikungunya fever around Indian subcontinent with emergence of *Aedes albopictus* as a most effective vector, *Aedes aegypti* also responsible for transmission of disease. NCVBDC, survey shows 59,535 suspects of Chikungunya in 2007; 95,091 in 2008; 73,288 in 2009; 48,176 in 2010; 20,402 in 2011; 15,977 in 2012; 18,840 in 2013; 16,049 in 2014; 27,553 in 2015 and 3342 confirmed cases in 2015. (13)

National Health Mission – Weekly Outbreak Report with district wise disease alert from January 2024 – 9 cases of DEN in Public health centre (PHC) Jamsar; 52 cases of CHIKV in Sontha village; 39 cases DEN in Government Hospital (GH) Changanassery; 5 cases and 1 death reported in PHC Vashind; 18 cases CHIKV in Puducherry taluk; 13 cases DEN in PHC Narivali; 9 cases CHIKV in Kapgal; 16 cases DEN in Khadkhad, In February 2024 – 18 cases Community Health Center (CHC) in Agali; 24 cases DEN in Bhairamgarth; 53 cases CHIKV in Nerebenchi; 9 cases DEN in Pangardarwadi; 9 cases CHIKV in Jashapar; 10 cases CHIKV in Govind Thanda; 6 cases and 1 death reported DEN in Panvel, In March 2024 – 16 cases in Family Health Center (FHC) Kunnukara; 88 cases DEN in Kalathur; 10 cases DEN in CHC Urngattiri; 5 cases DEN in PHC Panchincholi; 10 cases CHIKV in PHC Dhoki; 17 cases of CHIKV in Settigera; 45 cases DEN in Hanganakatti; 26 cases of DEN in Pirayiri; 26 cases DEN in Wagha; 64 cases DEN in Dadapur; 6 cases and 1 death report DEN in Fatimanagar; 15 DEN cases in Hivarda; 9 CHIKV cases in PHC Bhore, In April 2024 – 23 DEN cases in Taluk Head Quarters Hospital (THQH) Adimali; 10 DEN cases in Chirakkadavu; 19 DEN cases in GH Irinjalakkuda; 5 DEN cases and 1 death reported in Civil hospital Alibag; 6 CHIKV cases in PHC Bhuye; 22 DEN cases in District Hospital (DH) in Beed; 36 DEN cases and 1 death reported in Mahatma Gandhi Institute of Medical Sciences (MGIMS) in Sevagram; 7 DEN cases in Kottayam; 24 DEN cases in DH Akola; 10 DEN cases in CHC Vandiperiyar; 28 DEN cases in DH Dewas; 24 CHIKV cases in All India Institute of Medical Sciences (AIIMS) in Bhopal; 6 DEN cases in Chandrapur; 6 DEN cases

in PHC Betmogra; 8 DEN cases in DH Alibag; 11 DEN cases in Government Medical College & Hospital (GMCH) in Akola; 14 DEN cases in PHC Pohudul; 10 DEN cases in District Public Health Laboratory (DPHL) in Gharmora; 5 CHIKV cases in DH Botad; 3 DEN cases in PHC Velancheri; 5 DEN cases in CHC Susner; 5 DEN cases in CHC Khirkiya; 11 DEN cases in Chiplana; 22 DEN cases in PHC Malsur; 11 CHIKV cases in PHC Jammu; 10 DEN cases in PHC Khatav, May 2024 – 27 DEN cases in AIIMS Bhopal, 11 CHIKV cases in National Institute of Virology (NIV) in Pune; 9 DEN cases reported in MGIMS in Wardha. (14)

Vaccines for Dengue virus including, live attenuated vaccines, chimeric live attenuated vaccines, inactivated vaccines, recombinant proteins and DNA vaccines were under different stages of clinical trials. Licensed vaccine CYT-TDV (Dengvaxia®) manufactured by Sanofi Pasteur with 25-59% efficiency against Dengue viruses. (15) There are another two vaccines under the Phase III, TAK-003 (DENVax, Takeda/Inviragen) had 73.3-85.3% efficiency among adolescents and children; LATV (TV003/TV005) manufactured by NIAD/Butantan/Merck. (16&17) many of vaccines were under Phase I trials – TDENV-PIV (WRAIR/FioCruz/GSK) an Inactivated adjuvanted vaccine; D1ME100/TVDV (NMRC) a DNA vaccine; V180 (DEN-80E) a recombinant subunit and DENV-1-LVHC an live-attenuated vaccine. (18-21) Vaccines for Chikungunya, an Inactivated CHIKV were the first vaccine candidate in 1970 and now-a-days lots of vaccines under clinical trials especially mRNA vaccines were demonstrated a huge success against COVID-19, mRNA-lipid nanoparticle (mRNA-LNP) vaccine were expressing CHIKV E2-E1 antigen that had induced humoral and cellular response in mice (C57BL/6). (22) female *Aedes aegypti* mosquitoes lay egg on the surface of the open containers and rain water stagnant, sanitary workers need to clear all the larvae to decline the transmission and spread of Dengue virus and Chikungunya virus. (23) Indian Council of Medical Research (ICMR), National Center for Vector Borne Diseases Control (NVBDCP), National Center for Disease Control and Central Insecticides Board & Registration Committee (CIBRC) proposed a “Common Protocol for Uniform Evaluation of Public Health Pesticides for use in Vector Control” in 2023. (24) In the period of 2020 and 2021, a COVID 19 pandemic period there is a decreased cases in compare with previous years cases of Dengue was reported by World Health Organization (WHO), although the data for the period was incomplete. (25)

Conclusion

It is concluded based on the present study that the proper testing system by the responsible department should be carried out for Dengue and related viral infection throughout year with most care. This will be of human useful to the respective authority to test the Dengue viral infection at rapid ate. To test the various strains responsible for infection. Thus there is a ample scope to educate the people prevent the one of the killer disease.

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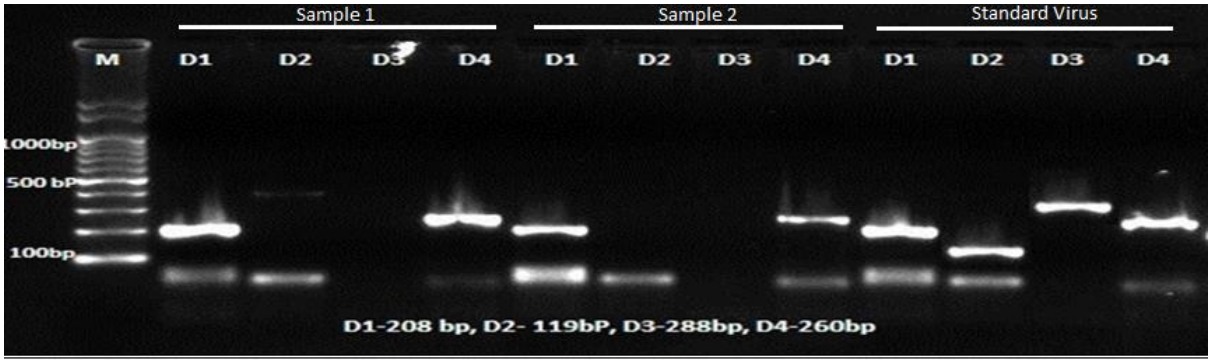

Figure 1. RT-PCR analysis for identification of Dengue virus from the patient samples collected from Karnataka. Lane M - DNA marker (100 bp), D1: Dengue 1, D2: Dengue 2, D3: Dengue 3, D4: Dengue 4, Standard virus D1: Dengue 1 (Positive control), Standard virus D 2: Dengue 2 (Positive control), Standard virus D3: Dengue 3 (Positive control), Standard virus D4: Dengue 4 (Positive control). Patient sample positive for Dengue 1 and Dengue 4.

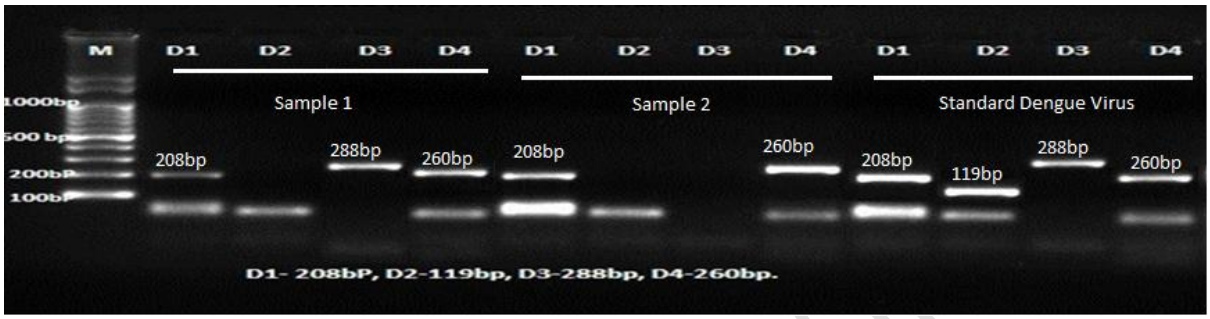

Figure 2. RT-PCR analysis for identification of Dengue virus from the patient samples collected from Karnataka. Lane M - DNA marker (100 bp), D1: Dengue 1, D2: Dengue 2, D3: Dengue 3, D4: Dengue 4, Standard virus D1: Dengue 1 (Positive control), Standard virus D 2: Dengue 2 (Positive control), Standard virus D3: Dengue 3 (Positive control), Standard virus D4: Dengue 4 (Positive control). Patient sample 1 were positive for Dengue 1, 3 and 4. Patient sample 2 were positive for Dengue 1 and Dengue 4.

Table 1: Total enrolment of screened samples male/female with positive percentage of Chikungunya, Dengue virus, Co-existence and Dengue serotypes.

	No of cases (positive %)	Male	Female
Total enrolment	547	305	242
Chikungunya virus	55 (10.1)	34 (61.8)	21 (38.2)
Dengue virus	32 (5.8)	16 (50)	16 (50)
Both Chikungunya and Dengue virus	1 (0.51)	-	1 (100)
Dengue Serotypes			
DENV 1	7 (21.8)	3	4
DENV 3	7 (21.8)	4	3
DENV 4	3 (9.4)	3	-
DENV 1 & 3	3 (9.4)	1	2
DENV 1 & 4	3 (9.4)	1	2
DENV 1,3 & 4	8 (25)	4	4
DENV 1 & Chikungunya	1 (3.1)	-	1
Total	**32**	**16**	**16**

Table 2: Analysis of positivity in age groups.

	0 – 10	11 - 20	21 - 30	31 - 40	41 - 82
Total enrolment	129 (23.6)	22 (4.0)	85 (15.5)	83 (15.2)	228 (41.7)
Chikungunya virus	11 (20)	1 (1.8)	6 (10.9)	10 (18.2)	27 (49.1)
Dengue virus	4 (12.5) in Infants only	3 (9.4)	9 (28.1)	6 (18.7)	10 (31.3)
Both Chikungunya & Dengue virus	-	-	-	1 (100)	-

Table 3: Month wise positivity rate for Chikungunya and Dengue viruses

Months	Chikungunya	Dengue
January	1 (1.8)	-
February	-	-
March	1 (1.8)	5 (15.6)
April	-	1 (3.1)
May	1 (1.8)	3 (9.4)
June	7 (12.7)	1 (3.1)
July	15 (27.3)	10 (31.3)
August	11 (20)	4 (12.5)
September	7 (12.7)	7 (21.9)
October	6 (10.9)	1 (3.1)
November	4 (7.3)	1 (3.1)
December	3 (5.5)	-

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