Co-Circulation of Dengue Serotypes in Coastal Andhra Pradesh, India - A Descriptive Study

Suryamani Chintapalli¹, Apparao Peddepalli², Sivajyothi Pilli³, Monika Deepthi Pilli⁴, Kanaka Mahalakshmi Yandra⁵

¹Department of Microbiology, Andhra Medical College, Visakhapatnam, Andhra Pradesh, India. ²Department of Microbiology, Andhra Medical College, Visakhapatnam, Andhra Pradesh, India. ³Department of Obstetrics and Gynaecology, Andhra Medical College, Visakhapatnam, Andhra Pradesh, India. ⁴Department of Microbiology, Andhra Medical College, Visakhapatnam, Andhra Pradesh, India. ⁵Department of Medicine, Andhra Medical College, Visakhapatnam, Andhra Pradesh, India.

ABSTRACT

BACKGROUND

Dengue is an acute febrile illness caused by mosquito-borne dengue viruses (DENV S) consisting of four serotypes (DENV 1 - 4) from flaviviridae family, genus flavivirus. These four are antigenically related serotypes designated as DEN V - 1, DEN V - 2, DEN V - 3 and DEN V - 4. In this context, the present study focuses on the circulating serotypes of dengue in coastal Andhra Pradesh.

METHODS

Study was done at Andhra Medical College, Visakhapatnam, teaching hospital in Andhra Pradesh. Acute phase dengue serum samples were collected and tested for NS1 antigen and antihuman IgM antibodies by enzyme linked -immunosorbent assay (ELISA). NS1 positive samples were further serotyped by reverse transcriptase real time polymerase chain reaction (R RT - PCR).

RESULTS

A total of 796 serum samples were included in the study. 300 (37.7 %) samples were positive for NS1 and IgM antibodies. 192 NS1 antigen positive samples were further processed for serotyping by r RT PCR. Among these samples 72 were negative by r RT PCR. DENV-2 (41 %) was the predominant serotype followed by DENV-4 (37 %), DENV-3 (12 %) and DENV-1 (10 %) in the descending order.

CONCLUSIONS

All the four dengue serotypes are in co-circulation. Among all the four types, DENV-2 was predominant, followed by DENV-4. By knowing the predominant serotype in circulation, we can forecast dengue outbreaks and take necessary measures like control of vectors.

KEY WORDS

Andhra Pradesh, Dengue Virus, Dengue Virus - 2, Dengue Virus - 4, Outbreak, Serotypes

Corresponding Author:

Dr. Chintapalli Suryamani,
Door no. 50-14-5/2/1, Gayatrinagar,
Beside Bhoomatha Gayatri Nivas,
Visakhapatnam-530016,
Andhra Pradesh, India
E-mail:
suryamanichintapalli27@gmail.com

DOI: 10.14260/jemds/2020/650

How to Cite This Article:

Chintapalli S, Peddepalli A, Pilli S, et al. Co -
circulation of dengue serotypes at coastal
Andhra Pradesh, India, a descriptive study.
J Evolution Med Dent Sci 2020;9(40):2965-
2969, DOI: 10.14260/jemds/2020/650

Submission 16-05-2020,
Peer Review 25-08-2020,
Acceptance 31-08-2020,
Published 05-10-2020.

Copyright © 2020 Suryamani Chintapalli et
al. This is an open access article distributed
under Creative Commons Attribution
License [Attribution 4.0 International (CC
BY 4.0)]

BACKGROUND

Dengue fever also known as “break bone” fever is an infectious tropical diseases caused by dengue virus (DEN V). It is one of the important mosquito borne viral infections of public health concern. All the four serotypes can cause clinical manifestations ranging from mild self - limiting illness to severe dengue haemorrhagic fever (DHF) and dengue shock syndrome. However, severity in dengue viral infection is known to be affected by secondary infection with heterologous antibodies (Abs) or with certain DEN V serotypes and genotypes. The changing pattern of dengue serotypes in a geographic location necessitates the continuous molecular surveillance of the circulating serotypes.

Dengue infection has emerged as a critical threat to population health globally over the past 50 years. The World Health Organization (WHO) estimates that 50 - 100 million dengue infection occur each year. The emergence and spread of four DENVs (serotypes) represent a global pandemic. The four distinct serotypes are namely DENV-1, DENV-2, DENV-3, and DENV-4.¹ In India, the first evidence of occurrence of dengue fever (DF) in the country was reported during 1946 from Madras (now called (Chennai) in Tamil Nadu.² The first dengue haemorrhagic fever (DHF) outbreak occurred in Calcutta (West Bengal in 1963.³ and subsequently numerous outbreaks have been reported.¹ all these reports suggest that India represents 33 % of the worldwide DENV infections.^{4,5} All the four serotypes i.e. DENV-1,2,3 and 4 have been isolated in India.³ and were co - circulating or concurrent infections were observed, which makes India one of the hyperendemic countries in the world.^{4,6,7} with frequent outbreaks.

Dengue infection have spread throughout the country with all serotypes in circulation without clear geographical distribution.² Serotyping of dengue is not performed routinely - therefore, data on circulation of different serotypes in various parts of the country are limited.

From South India, serotyping has been reported first from Tamil Nadu.² Few reports from Karnataka, Kerala and Telangana are also available. To the best of our knowledge research articles on serotyping of dengue from this part of Andhra Pradesh are not available from recent past. In this context the present.

METHODS

This descriptive study was conducted at the Department of Microbiology, Andhra Medical College / King George Hospital (KGH), a tertiary care teaching hospital in Visakhapatnam, Andhra Pradesh, India. Institute Ethics Committee approval was obtained for the study. All the patients clinically suspected to have dengue fever as per National Vector Borne Diseases Control Programme.² case definition presenting to the emergency room, medical outpatient departments, Medical wards and Medical intensive care units, paediatric medicine wards, Paediatric Medicine Intensive Care Units at KGH a Tertiary care Teaching Hospital, and also from Infectious Diseases.

Hospital which is located in GHCCD, Government Hospital of Chest and Communicable Diseases, Visakhapatnam. The

study was conducted from June 2019 to October 2019. Outbreak was included in the study.

Sample Collection

Suspected dengue patients of all age groups with 5 days and less duration of fever were included in the study. Peripheral venous blood (2 - 3 mL) was collected.⁸ The blood was allowed to clot at room temperature (20 – 25 °C) and then centrifuged at 330 rpm for 10 min,⁹ if not tested within 2 days, the separated serum was transferred to a sterile vial and stored frozen at – 70 °C,¹⁰ The cases were classified into dengue (with or without warning signs) and severe dengue according to the WHO¹¹ based on patients clinical presentations (only for serotyped cases)

Enzyme-Linked Immuno-Sorbent Assay

Serum was tested for the presence of NS1 antigen and antihuman IgM antibodies using Panbio Dengue Early enzyme – linked immunosorbent assay (ELISA) kit (Standard Diagnostics, Inc., Republic Korea) and National Institute of Virology (NIV) DEN IgM capture ELISA kit supplied by NIV, Pune, respectively, by ELISA as per the manufacturer's protocol. Samples tested positive for NS1 antigen were further processed for sero typing.

Dengue Serotyping

Viral RNA from NS1 antigen ELISA positive serum samples was extracted using Q1Aamp Viral RNA mini kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. The extracted RNA was reverse transcribed and real - time polymerase chain reaction (RTPCR) was done using Superscript - III one step RTPCR kit (Thermo Fisher Scientific Inc. Waltham, Massachusetts, united states). Sero typing was done using CDC DEN - 1 - 4 Real Time RT - PCR Assay for detection and sero type identification of DENV primers¹² primer probe set provided by CDC, Atlanta, USA, The reporting dyes used were DENV-1 (FAM), DENV-2 (VIC), DENV-3 (TEXAS RED), DENV-4 (CY5) and Rnase P (endogenous control) (FAM). Briefly the amplification conditions were reverse transcriptase - 30 min at 50 °C, Taq polymerase activation - 2 min at 95 °C 15 s at 95 °C 1 min at 60 °C for 45 cycles (after Taq polymerase activation steps). The results were interpreted as per kit instructions and analysed.

Statistical Analysis

Based on age patients were divided in to three groups; paediatrics < 18 years, adults 19 - 59 years and geriatrics > 60 years. Normally distributed continuous variables were summarized by mean and standard deviation. Remaining variables were summarized as median (interquartile range). All categorical variables were summarized as percentages. The association between categorical variables was studies by Pearson's Chi - square test. For data analysis, statistical software IBM SPSS Statistics 20.0 for windows version, (NY IBM Corp., USA) was used.

RESULTS

A total of 796 dengue suspected samples were received during the study period. Of these 796 samples, 300 (37.7 %) samples were positive for NS1 antigen and / or Ig M ELISA. Of these 300 positive samples, 210 were positive for dengue IgM antibodies and 192 for NS1 antigen (Table 1). Samples positive for both NS1 and IgM antibodies were 102. NS1 positive but IgM negative samples were 90 whereas 108 samples were IgM positive and NS1 negative. Age wise test results for NS1 antigen and IgM ELISA are shown in table 2.

Test	IgM +	IgM -
NS1 +	102	90
NS1 -	108	496

Table 1. Dengue NS1 and IgM Positive Cases

NS1+: NS1 Positive samples; NS1 -: NS1 negative samples; IgM+: IgM positive samples; IgM -: IgM negative samples.

Adults had the highest number of clinically suspected (406, 51 %) and laboratory confirmed cases of dengue (158, 52.7 %) with a mean age of 35.5 and found to be significant ($p = 0.037$). The next age group was paediatric a mean age of 8.8 tailed by geriatric group with 68.6 years mean age. No gender difference ($p = 0.569$) with positivity was observed in the study. The male to female ratio in the suspected dengue cases was 1.02:1 and in dengue positive cases was 0.95:1.

Age (in Years)	Number of Cases (%)	NS1 AND / OR IgM Positive (%)	Negative Cases	P
< 18	308 (38.7)	126 (42)	182 (36.7)	0.037
19 - 59	406 (51)	158 (52.7)	248 (50)	
> 60	82 (10.3)	16 (5.3)	66 (13.3)	
Total	796	300	496	

Table 2. Age Wise Distribution of Dengue Cases

A total of 192 NS1 positive samples were serotyped by r RT - PCR. The results are depicted in Table - 3 and gender wise distribution of serotypes in Table - 4. Of these only 120 were positive by r RT - PCR with preponderance of DENV-2 serotype. Negative result for DENV does not preclude infection¹² it may be because of low virus number or viral load in patient, being beyond detectable limit of assay.

Dengue Serotype	Number of Cases (%)	< 18 Years (%)	19 - 59 Years (%)	60 Years (%)
DENV-1	12 (6.3)	6	6	0
DENV-2	50 (26)	32	18	0
DENV-3	14 (7.3)	2	12	0
DENV-4	44 (22.9)	16	28	0
Negative	72 (37.5)	40	28	4
Total	192	96	92	4 (2.1)

Table 3. Distribution of Dengue Serotypes

The male - to - female ratio among dengue serotyped samples was 0.94:1. The distribution of serotypes is shown in Figure - 1. Nearly 51.67 % (DENV-1 six cases, DENV-2 thirty-two cases, DENV-3 four cases and DENV-4 twenty cases) of cases were dengue fever without warning signs. Almost 43.33 % were dengue fever with warning signs (DENV-1 six cases, DENV-2 eighteen cases, DENV-3 six cases, and DENV-4 twenty-two cases). Almost 5 % DENV-3 four cases and DENV-4 two cases were severe dengue with warning signs.

Dengue Serotypes	Number of Cases (%)	Female (%)	Male (%)	P
DENV-1	12 (6.3)	6 (5.9)	6 (6.7)	0.844
DENV-2	50 (26)	30 (29.4)	20 (22.2)	
DENV-3	14 (7.3)	6 (5.9)	8 (8.9)	
DENV-4	44 (22.9)	20 (19.6)	24 (26.7)	
Negative	72 (37.5)	40 (39.2)	32 (35.6)	
Total	192	102	90	

Table 4. Gender Wise Distribution of Dengue Serotypes

DISCUSSION

This descriptive study was done to identify the circulating dengue serotypes in this region. Southern part of India is pivotal for several viral diseases, amongst which the DENV tops the list. Recurrent dengue out breaks were experienced by South Indian states such as Telangana and Andhra Pradesh with increased disease severity, but regarding circulating serotypes very few studies are available.⁴ After 1965 outbreak report from Visakhapatnam,¹³ limited data are available from Andhra Pradesh, though studies from Hyderabad,¹⁴ (now in Telangana) are available.

Different parts of the country showed different trends of subtypes from time to time. In this study all four serotypes of dengue were reported. In South India during 1970 - 2000, DENV-2 was the principal serotype responsible for huge outbreak registered in 1996,^{2,14} which was slowly replaced by DENV-3. Later on, outbreaks with DENV-1, DENV-2 and DENV-3 became common. After 1970, DENV-4 was seldom reported from India.¹⁴ In 1963 DENV-1, DENV-2 and DENV-4 were isolated during an outbreak of dengue fever in Vellore, Tamil Nadu and all four serotypes were isolated in subsequent outbreaks,^{2,15} DENV-3 and DENV-4 were reported from Hyderabad during the outbreak of 2007: however, the circulation of all four serotypes was noted in the same place during 2014.^{4,16} The dominance of DENV-2 was reported from Karnataka, whereas three serotypes DENV-1, DENV-2, and DENV-3 and no cases due to DENV-4 were reported from Kerala.^{17,18} In Northern India studies from Delhi have reported all circulating serotypes.^{2,19}

During 2003 outbreaks, DENV-3 was predominant and co-circulated with DENV-1 in 2006 in Delhi.² In 2013 DENV-2 subjugated the outbreak with few cases of DENV-1 and DENV-3.¹⁹ A 4 year (2009 - 2012) study from Uttar Pradesh reported three DENV serotypes (DENV-1, 2, and 3), but the DENV-4 was not detected at all. The prevalence of each serotype has changed through the years, in 2009 DENV-3 was dominant serotype but in 2010 and 2011 DENV-2 became predominant which was later replaced by DENV-1.²⁰ In Eastern parts of India, studies from Odisha¹ and Kolkata reported DENV-2 and DENV-3 as the dominants circulating with no signs of DENV-4.^{1,21} A 3 year (2007 - 2010) study from Mumbai.²² Western India, reported all serotypes with DENV-2 as the predominant serotype, whereas DENV-3 was more common during 2012 - 2015. Co-circulation of DENV 1, 2 and 3 was reported from Pune.²³ during 2002 - 2008 with additional detection of DENV-4 serotype along others during 2009 - 2010.

Although circulation of all four DENV serotypes is reported, the dominant sub types and epidemiology of DENV keep on fluctuating. In our study all serotypes were detected with DENV-2 (41 %) as the dominant serotype. This is a concordance with other studies from Delhi,²² Uttar Pradesh,²³ and Mumbai.²² Although all dengue serotypes were circulating in different parts of the country DENV-4 infections were very few as compared to other serotypes. Even absence of DENV-4 was observed in some of the outbreaks such as reported from Odisha,¹ Kolkata,²¹ Uttar Pradesh,²⁰ and Delhi.¹⁹ In this study DENV-4 serotype was the common serotype only preceded by DENV-2 with 37 % positivity. Few cases of DENV-1 and DENV-3 were also detected. This was in contradiction to studies of Garg et al² and Mishra et al.²⁰

Adult age group was most affected amongst all the age groups. This was in concordance with studies from Mumbai,²² Delhi,²⁴ Odisha¹ and Uttar Pradesh.²⁰ This age group comprises working and student population. As dengue is mainly spread through Aedes mosquitoes which are day biters, the crowded areas such as workplaces and colleges may facilitate the spread of infection. Paediatric age group was next affected followed by geriatric age group. There was no significant association found in sero positivity between males and females. This was in opposition to studies from Mumbai,²² Delhi,¹⁹ Uttar Pradesh²⁰ and Northern Kerala,²⁵ where in males comprised a greater number of cases as compared to females. Gender based association of dengue sero type was not observed in our study.

Fever was the most common presentation affecting all patients (100 %). Other Complaints included myalgia, arthralgia, diarrhoea and vomiting. Haemorrhagic fever was associated with all serotypes infections with a high percentage in DENV-1 (50 %) and DENV-4 (50 %). Severe dengue was common in infections with DENV-3 and DENV-4, where jaundice, respiratory distress and elevated aspartate transaminase, and alanine transaminase levels were observed. Similar observations were made in another study.¹⁶ Impaired consciousness was also observed in DENV-4. Although DENV-2 was the predominant serotype in our study, the severity of infection was high in infections with DENV-3 and 4.

On testing with ELISA 108 samples were found to be NS1 antigen negative but IgM anti body positive. NS1 antigen can be detected up to 6 days of onset of symptoms, whereas IgM can be detected from day 4 of onset of symptoms.¹⁰ Low viral load or delay in reporting to the hospital may be one of the

possible causes for undetected NS1 antigen in these samples. As we have not done dengue IgG ELISA, we cannot conclude whether the infection was primary or secondary.

Despite the study having limitations in the form of short study period and small data, the findings about the distribution of serotypes are valuable. Nevertheless the trend of serotype dominance keeps on changing from time to time. Further continuous surveillance of dengue serotyping will provide the extent of distribution of different dengue serotypes. Further genotyping of serotypes should be done which can help in designing vaccines.

CONCLUSIONS

This study revealed the presence of four serotypes of dengue virus, with the predominance of DENV-2; however, severe infections were found in DENV-3 and 4. This information on predominant circulating serotypes helps us in forecasting dengue outbreaks, which will strengthen our ability and preparedness to address recurrent epidemics in this part of India.

Financial or Other Competing Interests: None.

REFERENCES

- [1] Rao MRK, Pandey RN, Das MK. Episodes of the epidemiological factors correlated with prevailing viral infections with dengue virus and molecular characterization of serotype-specific dengue virus circulation in Eastern India. *Infect Genet Evol* 2018;58:40-9.
- [2] Garg S, Chakravarti A, Singh R, et al. Dengue serotype specific seroprevalence among 5 to 10 year old children in India: a community based cross sectional study. *Int J Infect Dis* 2017;54:25-30.
- [3] Clinical Management of Dengue fever (DF) and Dengue Haemorrhagic Fever (DHF) New Delhi. National Vector Borne Diseases Control Programme 2008. Last accessed on 2018 Jan 13).
- [4] Vaddadi K, Gandikota C, Jain PK, et al. Co-circulation and co-infection of all dengue serotypes in Hyderabad, India 2014. *Epidemiol Infect* 2017;145(12):2563-74.
- [5] Bhatt S, Gething PW, Brady OJ, et al. The global distribution and burden of dengue. *Nature* 2013;496(7446):504-7.
- [6] Gupta E, Ballani N. Current perspectives on the spread of Dengue in India. *Infect Drug Resist* 2014;7:337-42.
- [7] Anoop M, Issac A, Mathew T, et al. Genetic characterization of dengue virus serotypes causing concurrent infection in an outbreak in Ernakulam, Kerala, South India. *Indian J Exp Biol* 2010;48(8):849-57.
- [8] Haryanto S, Hayati RF, Yohan B, et al. The molecular and clinical features of dengue during outbreak in Jambi, Indonesia in 2015. *Pathog Glob Health* 2016;110(3):119-29.
- [9] Klungthong C, Gibbons RV, Thaisomboonsuk B, et al. Dengue virus detection using whole blood for reverse transcriptase PCR and virus isolation. *J Clin Microbiol* 2007;45(8):2480-5.

- [10] World Health Organization. Comprehensive guidelines for prevention and control of dengue and dengue haemorrhagic fever. Revised and expanded edition. Regional Office for South-East Asia: 2011. Last accessed on 2018 Jan 13.
- [11] World Health Organization. Dengue: guidelines for diagnosis, treatment, prevention and control. New edition. Geneva. World Health Organization 2009.
- [12] CDCDEN-1-4 Real Time RT-PCR Assay for detection and serotype identification of Dengue virus. Centers for Disease Control and Prevention 2013. Last accessed on 2018 Jan 13.
- [13] Paul SD, Dandawate CN, Banerjee K, et al. Virological and serological studies on an outbreak of dengue-like illness in Visakhapatnam, Andhra Pradesh. *Indian J Med Res* 1965;53(8):777-89.
- [14] Dash PK, Sharma S, Srivastava A, et al. Emergence of dengue virus type 4 (genotype) in India. *Epidemiol Infect* 2011;139(6):857-61.
- [15] Chakravarti A, Arora R, luxemburger C. Fifty years of dengue in India. *Trans R Soc Trop Med Hyg* 2012;106(5):273-82.
- [16] Neeraja M, Lakshmi V, Dash PK, et al. The clinical, serological and molecular diagnosis of emerging dengue infection at a tertiary care institute in Southern India. *J Clin Diagn Res* 2013;7(3):457-61.
- [17] Vinodkumar CS, Kalpannavar NK, Basavarajappa KG, et al. Episode of coexisting infections with multiple dengue virus serotypes in central Karnataka, India. *J Infect Public Health* 2013;6(4):302-6.
- [18] Manakkadan A, Joseph I, Prasanna RR, et al. Lineage shift in Indian strains of dengue virus serotype-3 (Genotype-3), evidenced by detection of lineage IV strains in clinical cases from Kerala. *Virol J* 2013;10:37.
- [19] Afreen N, Deebea F, Naqvi I, et al. Molecular investigation of 2013 dengue fever outbreak from Delhi, India. *PLoS Curr* 2014;6:ecurrents.outbreaks.0411252a8b82aa933f6540abb54a855f.
- [20] Mishra G, Jain A, Prakash O, et al. Molecular characterization of dengue viruses circulating during 2009-2012 in Uttar Pradesh, India. *J Med Virol* 2015;87(1):68-75.
- [21] Sarkar A, Taraphdar D, Chatterjee S. Molecular typing of dengue virus circulating in Kolkata, India in 2010. *J Trop Med* 2012;2012:960329.
- [22] Shastri J, Williamson M, Vaidya N, et al. Nine year trends of dengue virus infection in Mumbai, Western India. *J Lab Physicians* 2017;9(4):296-302.
- [23] Cecilia D, Kakade MB, Bhagat AB, et al. Detection of dengue-4 virus in Pune, Western India after an absence of 30 years -- its association with two severe cases. *Virol J* 2011;8:46.
- [24] Bharaj P, Chahar HS, Pandey A, et al. Concurrent infections by all four dengue virus serotypes during an outbreak of dengue in 2006 in Delhi, India. *Virol J* 2008;5:1.
- [25] Reddy MN, Dungdung R, Valliyott L, et al. Occurrence of concurrent infections with multiple serotypes of dengue viruses during 2013-15 in Northern Kerala, India. *Peer J* 2017;5:e2970.