SEROTYPING OF DENGUE VIRUS USING REAL TIME REVERSE TRANSCRIPTASE PCR IN A TERTIARY CARE CENTRE IN SOUTH INDIA.

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ABSTRACT

INTRODUCTION- Dengue is a vector-borne disease caused by the dengue virus, belonging to the genus Flavivirus and family Flaviviridae. There are four antigenically related serotypes designated as DENV-1, DENV-2, DENV-3 and DENV-4. All the four serotypes can cause clinical manifestation ranging from mild self-limiting illness to severe dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). There is a need for constant molecular surveillance of the circulating serotypes in a geographical location due to the changing pattern of dengue serotypes.

MATERIALS AND METHODS- A total of 100 samples, positive for either of IgM Ab or NS1 Ag ELISA, were selected for the present study which was done at Department of Microbiology (BMCRI) & State Level Virus Research and Diagnostic Laboratory, Bangalore, during September and October 2017. Clinical data was collected at the time of sample collection. RNA purification was done by using Qiagen QiAMP RNA purification kit according to the manufacturer's instructions. The detection of Dengue virus serotypes was performed by using CDC DENV-1-4 Real-Time RT-PCR Assay kit.

RESULTS- A total of 30 samples showed positive amplification in Real time RT-PCR out of 100 samples tested. The assay could detect all the four circulating serotypes. Of the 30 positive samples, 6 (20%) were DENV-1 serotype, 10 (33.3%) were DENV-2 serotype, 9(30%) were DENV-3 serotype and 5 (16.7%) were DENV-4 serotype.

DISCUSSION -Dengue is a major public health problem with varied clinical manifestations.The existence of two or more serotypes during the same time period has been widely considered as one of the major cause of disease severity. However, a definite link between distinct serotypes and severe manifestations has not been established yet. This study shows the existence of all 4 serotypes with DENV-2 and DENV-3 being the predominant serotypes in and around Bangalore

during the study period. According to clinical data, it was observed that DENV-2 and DENV-3 had higher occurrence of severe clinical manifestations.

CONCLUSION- This study showed the presence of all four serotypes in this geographical region, with DENV-2 and DENV-3 as two predominant serotypes. Further it was observed that DENV-2 and DENV-3 serotype patients had higher occurrence of severe clinical manifestations.

Keywords : Dengue, Serotype, DENV, Viral haemorrhagic fever

INTRODUCTION:

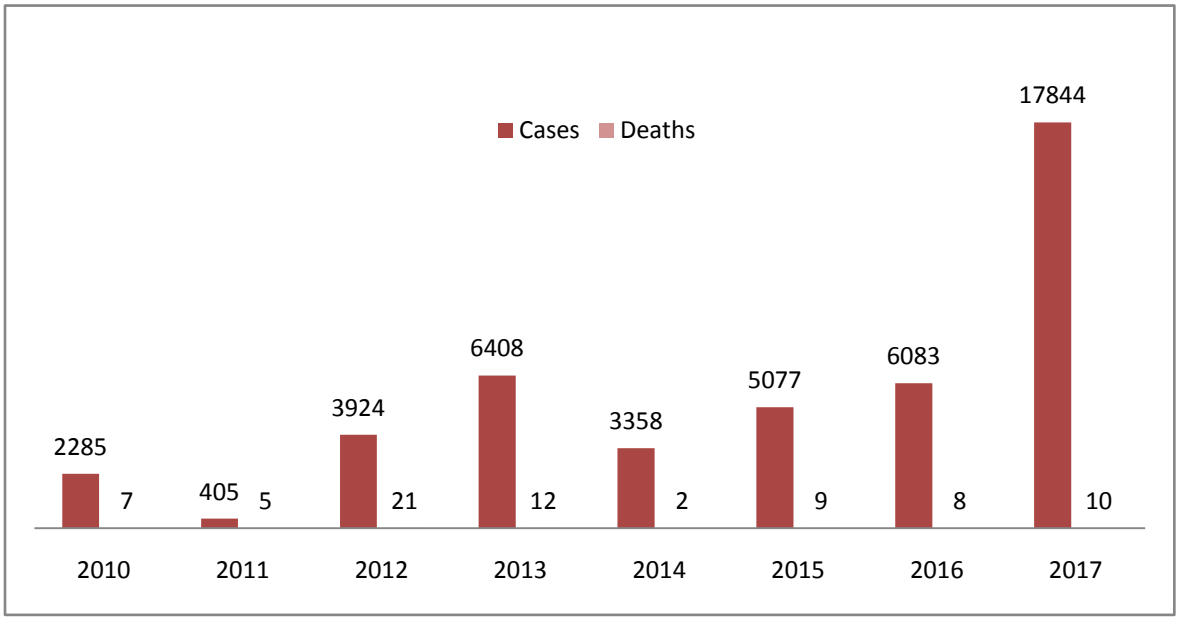
Dengue fever is a vector borne viral haemorrhagic fever caused by Dengue virus (DENV), belonging to genus Flavivirus and family Flaviviridae and transmitted by Aedes mosquitoes. It is a major public health concern with approximately two-fifths of the world populationbeing at risk of infection (5^{th}serotype)[1]. There are four closely related serotypes (DENV-1, DENV-2, DENV-3and DENV-4) that are antigenically and genetically distinct [3]. A fifth serotype, DENV-5, has been identified in October-2013 (5^{th} serotype).

The genome of DENV is a linear, nonsegmented, positive-sense strand of RNA of approximately 10.6– 11 kb, which translates into seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) and three structural proteins (capsid, premembrane, and envelope)[2]. The four DENV serotypes share 65–70% sequence homology and are further clustered into different genotypes on account of high mutation rates(genome of dengue).All 4 serotypes can cause a wide spectrum of clinical manifestations from self limiting febrile illness to severe Dengue hemorrhagic fever (DHF) and Dengue shock syndrome (DSS)(clinical manifest).The seroprevalence of Dengue differs geographically in India.

According to a study conducted by Murhekar etal, the seroprevalence was high in the southern (76·9%), western (62·3%), and northern (60·3%) regions. Urban areas (70.9%) show higher prevalence as compared to rural areas (42.3%). The disease

burden (cases and deaths) in Karnataka since 2010 as shown in diagram-1 suggests an increasing trend in number of cases[4].This increasing number of cases of dengue thus needs a constant molecular surveillance of the circulating serotypes in a particular geographical location. Real Time Reverse Transcriptase PCR is highly sensitive and specific for detection, quantitation and serotyping of Dengue virus[4].

Diagram-1(Disease burden in Karnataka 2010-2017)[4]



OBJECTIVES:

To detect the circulating serotype of dengue virus using Real Time RT-PCR in samples from patients attending a Tertiary Care Centre in Bangalore.

MATERIALS and METHODS:

A total of 100 serum samples, positive for either of IgM Ab or NS1 Ag ELISA, were collected. Storage of serum samples was done at -20^{0}C. The study was conducted at Department of Microbiology (BMCRI) & State Level Virus Research

and Diagnostic Laboratory, Bangalore, during September and October 2017.Relevant Clinical data was collected at the time of sample collection. Manual RNA purification was done by using Qiagen QiAMP Viral RNA Mini Kit according to the manufacturer's instructions. The extracted RNA was subsequently processed by Real time PCR. In case of expected delay in processing, the extracted RNA was stored at -80^{0}C. Stored samples were thawed to room temperature before processing in Real time PCR. Repeated freeze thaw cycles were avoided. The detection of Dengue virus serotypes was performed by using CDC DENV 1-4 Real-Time Reverse Transcriptase-PCR Assay kit.The Assay was run in multiplex format reaction (the four DENV serotypes were identified in the same reaction) according to the kit insert protocol. Thermal cycler used was BIO-RAD CFX96 Real Time system C1000. The data was analyzed by using descriptive statistics. Data were described on the basis of numbers and percentages.

RESULTS:

Out of 100 serum samples, 56 were from male patients and 44 were from female patients. 21 samples belonged to 0-18 yrs age group, 63 samples belonged to 19-60 yrs and 16 samples belonged to age group.

A total of 30 (30%) samples showed positive amplification in Real time RT-PCR out of 100 samples tested. The assay detected all the four circulating serotypes. Of the 30 positive samples, 6 (20%) were DENV-1 serotype, 12 (40%) were DENV-2 serotype, 10 (33.3%) were DENV-3 serotype and 2 (6.7%) were DENV-4 serotype as shown in diagram-1.The PCR amplification curve is shown in diagram-2. The occurrence of clinical manifestations at the time of admission with the corresponding serotypes is shown as in Table-1.

Diagram-2 (Circulating Serotypes in this study)

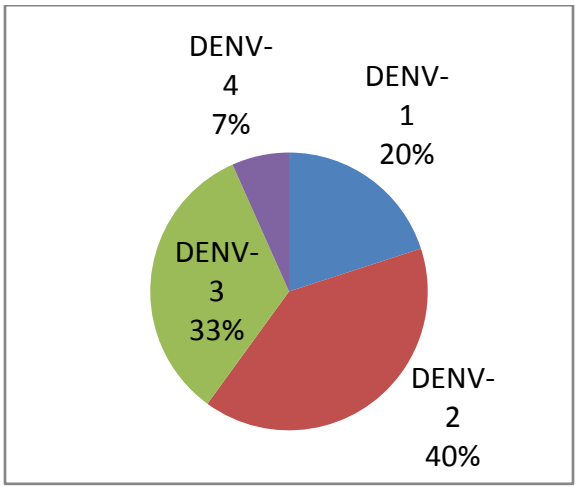
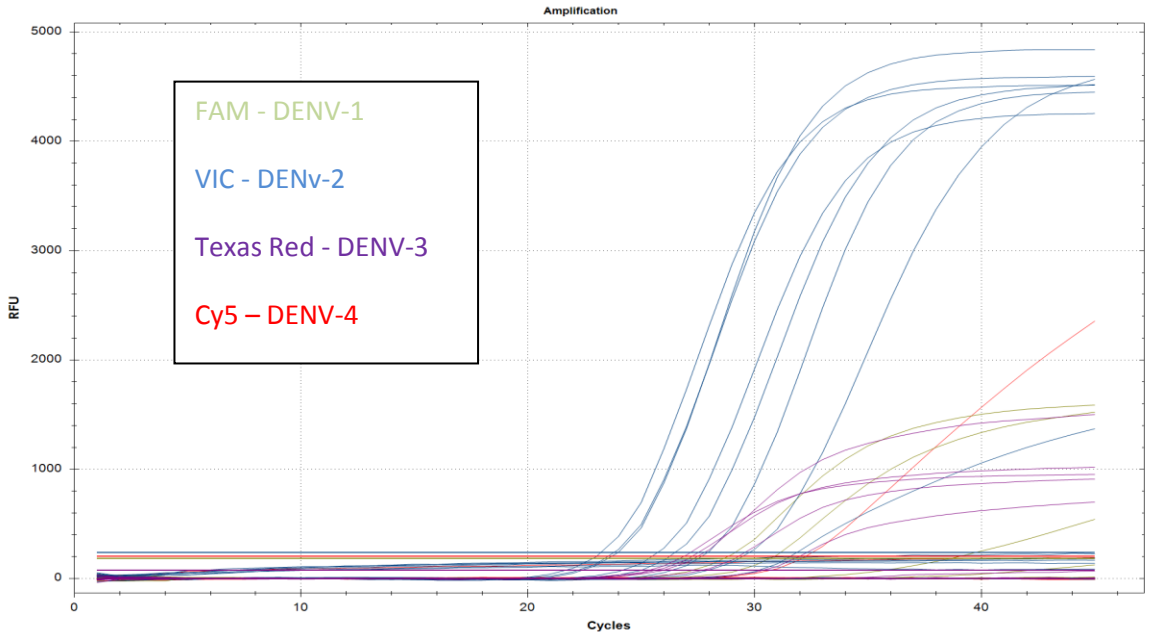


Table-1 (Clinical manifestations with corresponding serotypes)

Disease manifestation	DENV-1 (6)	DENV-2 (12)	DENV-3 (10)	DENV-4 (2)
FEVER	6 (100%)	12 (100%)	10 (100%)	2 (100%)
RETRO ORBITAL PAIN / HEADACHE	3 (50%)	6 (50%)	7 (70%)	2 (100%)
ABDOMINAL PAIN	2 (33.3%)	10 (83.3%)	3 (30%)	1 (50%)
ARTHRALGIA	2 (33.3%)	12 (100%)	5 (50%)	1 (50%)
MYALGIA	1 (16.7%)	8 (66.6%)	3 (30%)	1 (50%)
BLEEDING MANIFESTATIONS	0	3 (25%)	0	0
DENGUE SHOCK SYNDROME (DSS)	0	1 (8.3%)	0	0

Diagram-3 (Real Time PCR Amplification curve)



DISCUSSION:

Dengue is a major public health problem with varied clinical manifestations ranging from mild asymptomatic illness to viral hemorrhagic fever. The existence of two or more serotypes during the same time period has been widely considered as one of the major cause of disease severity. However, a definite link between distinct serotypes and severe manifestations has not been established yet[8].

This study shows the existence of all 4 serotypes with DENV-2 and DENV-3 being the predominant serotypes in and around Bangalore during the study period. According to clinical data, it was observed that DENV-2 and DENV-3 had higher occurrence of severe clinical manifestations. Present study is in concordance with other studies done as shown in Table-3.

Vinodkumar C S et al., studied 72 dengue cases in Davangere district in Karnataka where they found 42 cases with all four serotypes circulating in the region in which Den-2 was the predominant serotype found.[6]

Damodar T et al., studied 83 dengue cases of which, 33 cases were subjected for Real time PCR in which Den 1, 2 and 3 serotypes were found and Den -2 was reported to be the predominant serotype, followed by Den-3.[7]

Yergolkar et al., Kumaria R et al., in 2016 and 2010 respectively, found Den-2 and Den-3 to be the most common serotypes.[8][9]

Shah PS et al; have said that DEN-2 and DEN-3 were the serotypes circulating in the samples tested.[10]

Table 2: Comparison of different studies comparing the serotypes.

Sl no.	Authors	Place of Study	Year	Results
1.	**VinodKumar *et al [5]***	Davangere	2013	DENV-2 dominated the outbreak, followed by DENV-3
2.	**Damodar *et al [6]***	Mangalore	2013/14	DENV-2 was found to be the predominant serotype followed by DENV-3 and DENV-1
3.	**Yergolkar*et al [7]***	Bangalore	2016	6 genotypes of DENV-2 (predominant) and 3 genotypes of DENV-1 serotype found to be circulating
4.	**Kumaria R *et al [8]***	New Delhi	2010	DENV-2 and DENV-3 as two predominant serotypes. The clinical manifestations, such as abdominal pain arthralgia, hepatomegaly, were higher in Den-2 patients.
5.	**Shah PS *et al [9]***	Pune	NIV annual report 04/05	DENV-2 and DENV-3 were the only serotypes circulating out of the 48 samples tested.

CONCLUSION

This study showed the presence of all fourserotypes in this geographical region,withDENV-2 and DENV-3 as two predominant serotypes.Further it was observed that DENV-2 andDENV-3 serotype patients had higheroccurrence of severeclinical manifestationslike abdominal pain, arthralgia, myalgia andbleeding instances.

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