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Dengue and Viral Circulation in Infected Cases of Ahmedabad: A Single Centre Study

Parth S. Shah[1,2], Nidhi D. Shah[1,3], Ayushi S. Patel[1], Siddhi M. Kurtadikar[1], Hemangi D. Dixit[1], Khusbhu R. Patel[1], Shiva M. Murarka[1], Bhavini S. Shah[1] and Mandava V. Rao[1,4]

[1]Department of Molecular Genetics, Supratech Micropath Research Laboratory, Ahmedabad, Gujarat, India
[2]Department of Medicine, Lahey Medical Center, Boston, MA, USA
[3]Department of Pediatrics Nassau University and Medical Centre, New York City, NY, USA
[4]School of Sciences, Gujarat University. Ahmedabad, Gujarat, India

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ABSTRACT The dengue fever is a debilitating arthropod-borne disease caused by one of the four closely related dengue viruses. The symptoms appear 3 – 14 days after mosquito bite and range from mild fever to very high fever. Based on these symptoms, a total of 178 referral cases were analyzed. Patients were asked to fill out a consent form which was followed by blood collection. Parameters that were analyzed amongst these patients include: Platelet count, transaminases, Dengue Real Time-PCR detection, gel-based dengue serotyping. An overall 12.36 percent (22/178) prevalence of dengue infection was detected in the post-monsoon season that is from October 2016 to March 2017. Younger age groups of males were more affected (18.2%) amongst all groups. Patients who tested positive for dengue had markedly reduced platelet counts as compared to those of negative control cases. Altered serum glutamic oxaloacetic transaminase (SGOT) levels were also observed amongst patients with infection leading to liver dysfunction. The most common serotype prevalent was DENV-3 (45%), followed by DENV-4 (36%) and then DENV-2 (18%). No prevalence of DENV-1 was found. In the absence of targeted vaccination and medication for dengue fever, it is essential to study the epidemiology of it for controlling the spread of dengue during post-monsoon season.

INTRODUCTION

Dengue and Chikungunya are vector borne diseases and are prevalent in tropical regions of Africa and Asia including India for decades. Dengue, arbovirus infection caused by four serotypes (DENV1 – 4) that produce the disease ranging from self-limiting dengue fever (DF) to server life-threatening dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). Each one has its own unique characteristic and manifestation depending upon its interaction with the host's response (Lee et al. 2012; WHO 2010; Islam et al. 2016; Garg et al. 2017). The global prevalence of dengue estimated is 2.5 billion people at risk of acquiring this viral infection and more than 50 x 10^6 new viral infection are being projected annually (Chakravarti et al. 2016). In Gujarat, the report on dengue infection is revealed that an outbreak of it occurred in western state of Gujarat and Rajasthan in addition to North, South, and East Indian states. More males of young who are active are prone to suffer dengue ranging from asymptomatic or mild febrile illness to severe form (Patankar et al. 2014; Mehta et al. 2014; Gupta and Banal 2017) detected by following variety of methods. However, no report so far is available on viral circulation of dengue in cases of Gujarat and their distribution among infected cases, though other places DENV 1- 4 serotypes distribution during dengue infection was well attempted (Gupta et al. 2012). Islam et al. (2016) reported in post monsoon season of 2015 in Delhi, where high incidence of DENV-2 (66.66%) followed by DENV-1, DENV-3 and DENV-4 was observed in their study. Hence, this report in our centre is presented after complete investigation of referral dengue cases during post-monsoon season of 2016 – 2017.

METHODOLOGY

Patient Selection

Patients (178) suspected for this fever based on clinician's report were referred to the researchers' centre from various areas in Ahmedabad (Maninagar, Paldi, S.G. Highway, Navrang-

pura) from October 2016 to March 2017. The patient was asked to fill out a consent form followed by blood collection that was used for analysis in this study. This project was approved by Human Ethical Committee (HEC) of Gujarat University (GUHEC-001-2015) for investigation.

Haematological and Biochemical Profile

A platelet count was done using cell counter (Siemen's Advair 2010i, Germany). Serum transaminases that were estimated in this study included serum glutamic oxaloacetic transaminase (S.G.O.T)/Aspartate transaminase (AST) and serum glutamate-pyruvate transaminase (S.G.P.T)/Alanine transaminase (ALT) using a kit (Dimension EXL, Germany) that were expressed as U/L and compared.

Viral RNA Extraction

Viral RNA was extracted from EDTA plasma samples using the Automated Perkin Elmer Viral RNA/DNA 200 extraction kit (Perkin Elmer,) or QIAamp® Viral RNA mini kit (Qiagen, Germany) as per manufacturers' instruction.

Dengue Virus Detection

The Real Time-PCR assay used in this study for the detection of the dengue virus was RealStar® Dengue RT-PCR Kit 2.0 (Altona Diagnostics). The assay allowed for a qualitative detection of dengue virus specific RNA, with reverse-transcription of the RNA into complementary DNA (cDNA) followed by PCR for the amplification of specific target sequences. The probes utilized were specific for DENV RNA labeled with the fluorophore FAM™ and specific for the Internal Control (IC) is labeled with the fluorophore JOE™.

Dengue Virus Serotyping

For those patients who tested positive for dengue, virus serotype was determined using reverse-transcription gel-based PCR. The extracted RNA was transcribed into cDNA using Takara Clonetech 1st Primer Synthesis cDNA kit as per manufacturer's instructions. First an external PCR carried out to amplify the CprM region using published primers (Islam et al. 2016). The first PCR amplifies a 511bp segment of the dengue virus specific to all four serotypes. The external PCR assay was a 25 µl reaction assay composed of 12.5 µl Go Taq Green Mastermix (2X), 0.24 µM of forward and reverse primers (D1 and D2), 8.9 µl of NFW and 3.0 µl of cDNA. The PCR cycling conditions were carried out as follows: 94°C for 2 minutes, 35 cycles of 94°C for 45s, 52°C for 30s and 72°C for 60s and a final extension at 72°C for 10 minutes. Followed by a semi -nested PCR was carried out to amplify serotype specific regions using published primers. A 25 µl PCR assay composed of 12.5 µl of Go Taq Green Master Mix (2X), 0.4 µM of each primer D1, TS1, TS2, TS3, and TS4, 7 µl of NFW and 3.0 µl of diluted (1:5) external PCR product. The PCR cycling conditions were carried out as follows: 94°C for 1 minute followed by 25 cycles of 94°C for 30s, 54ÚC for 30s and 72°C for 1 minute and a final extension at 72°C for 10 minutes. The amplicon size for DENV-1 was 482bp, 119bp for DENV-2, 290bp for DENV-3 and 392bp for DENV-4 (Islam et al. 2016). The PCR products were then run on a two percent agarose gel stained with ethidium bromide and observed under UV light.

RESULTS

Amongst the 178 referred cases at the researchers' centre from October 2016 to March 2017 prevalence rate of 12.36 percent (22/178) was detected for dengue. The percent of seropositive patients ranged from 0 to 9.55 percent within these six months. Maximum cases were detected positive in the month of October (9.55%) followed by November (2.25%) and December (0.56%), in other months, that is, January to March 2017 no case was detected (Table 1).

Table 1: Total prevalence of DENV in cases of six months (2016 – 2017)

Month	*Total*	*Affected*	*Percent affected*
October	57	17	9.55
November	66	4	2.25
December	24	1	0.56
January	18	0	0
February	9	0	0
March	4	0	0
Total	178	22	12.36

Amongst four age groups viz. 0-20, 21-40, 41-50 and 51 and above year age groups, the positiv-

ity decreased with age where the highest percent was in youngsters (22.5%) and further males were more affected (6.7%) in the six months (Table 2).

Table 2: Group-wise DENV cases in the study (October 2016 – March 2017)

Age group (years)	*Male*	*Female*	*Total positive*
0-20 (22)	4 (18.2%)	1 (4.5%)	5 (22.7%)
21-40 (52)	6 (11.5%)	3 (5.8%)	9 (17.3%)
41-50 (23)	0 (0.0%)	4 (17.4%)	4 (17.4%)
51 and above (81)	2 (2.5%)	2 (2.5%)	4 (5%)
Total (178)	12 (6.7%)	10 (5.6%)	22 (12.4%)

Blood Platelet Cell Count and Transaminase Levels

The SGPT/SGOT levels were significantly ($p<0.05$) higher in 0 to 20 and 41 to 50 age group. Additionally, SGOT/SGPT levels were significantly ($p<0.01$) increased in all age groups being maximum in the 41 – 50 age group (349%). The normal range is 0 – 37 U/L. Platelet counts were significantly ($p<0.05$; $p<0.01$) decreased in all age groups being higher in early age groups (Table 3).

Table 3: Blood cell count in dengue cases

Age group (years)	*SGPT 14 - 59 U/L (100%)*	*SGOT 0 - 37 U/L (100%)*	*Platelet Count 150000 - 500000 /ul*
0-20	193%	302%	120433±56288*
21-40	78%	N/A	126666±49303*
41-50	273%	349%	142000±60265+
51 & Above	N/A	N/A	141000±61582+

N.A. – Not Available; values are mean ±S.D; +- $p< 0.05$ *- 0.01

Serotype-Distribution

The most common serotype prevalent during these six months was DEN-3 (45%), followed by DEN-4 (36%) and then DEN-2 (18%). No prevalence of DENV-1 was found in Ahmedabad, this viral distribution differed DENV-3, DENV-4 followed by DENV-2 (Table 4, Fig. 1).

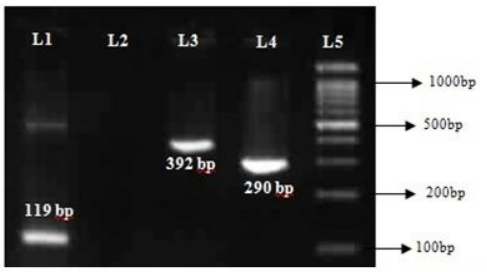

Fig. 1. Gel-image for dengue serotyping L1: Patient sample with dengue serotype 2 (119 b;); L2: Patient sample negative for dengue; L3: Patient sample with dengue serotype 4 (392 bp;) L4: Patient sample with dengue serotype 3 (290 bp); L5: 100bp Ladder

DISCUSSION

Dengue infection is one of the major health problems in India. It is transmitted to human by *Aedes aegypti* and *A. albopictus* mosquito. In this study, researchers' report 22 cases of dengue from referral cases of 178 in the post-monsoon season. These cases are identified by clinical manifestation according to WHO (2009) and Lee et al. (2012). The symptoms include fever, vomiting, rashes, nausea, aches and pains. Severe symptoms of DHF include bleeding in the researchers' patients. It is more in young age groups followed by old age as young adult population are active. These seemed to be more affected than females during post-monsoon season. Same observations are documented by earlier workers in all parts of the globe including India (Cecilia 2014; Bhatt et al. 2013; Gupta et al. 2012; Islam et al. 2016; Gupta and Bansal 2017; Saravanan et al. 2017). In Gujarat other researchers also obtained same results where young adult male population are affected in Gujarat (Patan-

Table 4: Location-wise distribution for dengue serotype

Location	*Serotype 1*	*Serotype 2*	*Serotype 3*	*Serotype 4*	*Total (%)*
Maninagar	-	1	3	3	7 (32%)
Paldi	-	1	4	2	7 (32%)
S. G. Highway	-	-	2	2	4 (18%)
Navrangpura	-	-	1	-	1 (5%)
Others	-	2	-	1	3 (14%)
Total	0 (0%)	4 (18%)	10 (45%)	8 (36%)	22 (100%)

kar et al. 2014; Mehta et al. 2014) during post-monsoon months, which is September, October, November months similar to these data.

The data was further supported by drastic decline in platelet count in this study as per recommendations of WHO (2009) and Lee et al. (2012) followed by bleeding in few severe DHF cases. Further transaminases (ALT and AST) in serum levels were also noted to be more in DF and DHF conditions in the study. This indicated liver dysfunction. It is also known that indices like protein, albumin and urea levels also increase during DF to separate chikungunya (Lee et al. 2012). In addition to higher haematocrit, tachycardia, cough and fever in dengue infected cases (Lee et al. 2012; Shah et al. 2017) were altered.

Gupta et al. (2006) on dengue infection of 2003 – 2005 in Delhi found co-circulation of all serotypes in 2003; later serotype 3 emerged as pre-dominant type in 2005. Bharaj et al. (2008) reported in their study of 69 samples of dengue. All the four were found being DENV-3 predominant type. In the study conducted by Chakravarti et al. (2012), overall DENV-2 and DENV-3 were most pre-dominant serotypes during 2003 – 2005. But DENV-1 replaced these strains in the year 2008. In Pakistan, DENV-2 was dominant in dengue samples collected during the period of these years (2007 – 2009). Recently, Gupta et al. (2012) mentioned that circulation of DENV-1 serotypes was pre-dominant in 2010 – 2011 after DENV-3 predominance earlier from a single centre hospital-based study in New Delhi. Findings of Islam et al. (2016) during post-monsoon season of the year in New Delhi detected all four serotypes in their study with 66.66 percent of samples positive for DENV-2, 22.22 percent for DENV-1 and 16.7 percent each for DENV-3 and DENV- 4. Co-infection with more than one serotype was also noticed in age group 21 – 30 years. Lastly, in this study the finding of seropositivity of viral circulation in post-monsoon indicated the DENV-3 had high incidence followed by DENV-4 and DENV-2. DENV-1 was absent in the 22 samples analyzed amongst the infected Gujarat population. Further, area wise of Ahmedabad city, the viral circulation was also variable as seen in the present study, where Paldi and Maninagar areas had maximum (32%) viral circulation.

All these data of viral circulation in dengue infected cases revealed that the type of virus changes over the years depending upon regions and post climatic conditions (Garg et al. 2017; Gupta and Banal 2017). Hence, these changes in genotype and mutations occur in dengue viral serotypes that provide information on their circulation in that region. Additionally correlation of the disease severity with different serotypes and concurrent infections may provide enrichment on the pathogenesis of this virus. Further surveillance and monitoring of the DENV serotype and their associated genotypes/sub-types can contribute to formulation of control measures.

CONCLUSION

The researchers' data clearly mentioned that dengue is infected during post-monsoon (2016 – 2017) and are related to age and sex. Hence, pre-monsoon measures are an urgent need to control this disease, in addition to vaccination and other health measures. Further, serotyping of virus circulation in Gujarat might help to understand the genotype and mutational changes occurring region wise to understand the spread of Dengue Fever (DF), Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) in addition to WHO recommendations of dengue and its controlling measures in developing countries like India.

RECOMMENDATIONS

This cohort indicates an urgent need to check dengue fever in Gujarat and care is to be taken by the respective health centres at various places to eradicate the viral disease causing mosquito sites and provide clean environment to the public.

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