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ORIGINAL RESEARCH

Molecular Characterization & Hematological Correlation Of Dengue Virus Among The Patients Attending Tertiary Care Centre, Jaipur, Rajasthan

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ABSTRACT

Introduction: Dengue is arboviral diseases spread by the bite of infected Aedes mosquito carrying these viruses. Every year, thousands of individuals are affected and contribute to the burden of health care. However, there was little information on serotypes of dengue virus circulating in this part of the country. This study was carried out molecular characterization of Dengue virus isolated from patients in our area and study correlation of prevalent serotype with severity of disease along with the hematological parameters.

Materials& Methods: 1192 dengue like illness patient samples were collected from 2018 -2020 at JNUIMSRC, Jaipur. RNA extraction was done by Qiagen viral RNA mini kit & followed byRTPCR by using type specific primers & probes were used for Dengue RT PCR.

Results: A total of 1192 dengue like illness cases who had less than <14 days of fever, blood samples were collected from various departments of JNUIMSRC, Jaipur. Out to 1192 cases 585 (49.07%) where Dengue was found. In Dengue Majority of the patients 161 males (27.52%) & 73(12.47%) females belonged to the 21-30 yrs of age group. Out of 585 cases total 108 (18.46%) were DEN I, 163 (27.86%) DEN II, 218 (37.26%) DEN III and 74 (12.64%) DEN IV and along with this 24-mix serotype infection was found.

Conclusion: Dengue remains to be an important health problem in India and across the globe. There is neither any specific treatment nor any effective vaccine for dengue so far available in India. So, the proper diagnosis is the first requirement for the

management of the disease. DHF &DSS are life threatening. Molecular method is much needed as it hardly takes 4 hrs. To produce accurate result
Key words: Dengue, Serotype, Dengue RT-PCR, Hematological parameters.

INTRODUCTION

Vector-borne disease is the major cause of human morbidity and mortality between 1700 and 2000, more than all other causes combined[1]. The incidence of people infected by mosquito vectors, in particular is over whelming. According to the World Health Organization (WHO) report, this small insect is accountable for a few million fatalities and many more morbidities each year[2]. The past decade has also seen epidemics attribute to the group of seven arthritogenic *Alphavirus*es belonging to the *Togaviridae* family[3,4]. Dengue infections are caused by four closely related serotypes named DEN I, DEN II, DEN III, and DEN-IV. The four dengue viruses are similar — they share approximately 65% of their genomes — but even within a single serotype, there is some genetic variation. Despite these variations, infection with each of the dengue serotypes results in the same disease and range of clinical symptoms[5].

It is crucial to determine which serotypes of Dengue virus are circulating since previous infection with one of the four Dengue serotypes can be an important risk factor for developing DHF-DSS upon secondary infection with a heterotypic serotype. In this study serotyping of Dengue viruses will be attempted in clinical samples. Every year, thousands of individuals are affected and contribute to the burden of health care. Based on the data of National Vector Borne Disease Control Programme (NVBDCP)[6]. The number of Dengue cases reported in 2015, 99913 cases were positive of dengue it which 220 patients were died, while total 129166 with 245 deaths were reported in 2016, in 2017 it was reached 188401 with 325 deaths, 101192 dengue cases with 172 deaths were reported in 2018, while in 2019, 136422 dengue cases were found all over India.[6]

However, there was little information on serotypes of dengue virus circulating in this part of the country. This study was carried out molecular characterization of Dengue virus isolated from patients in our area and study correlation of prevalent serotype with severity of disease along with the haematological & biochemical parameters.

MATERIALS& METHODS

Total 1192 Chikungunya & Dengue like illness patients who have less than 14 days of fever history were enrolled in this study from 2018-2020. All the samples were collected from the patients attending OPD, IPD & ICU at Jaipur National University Institute for Medical Sciences & Research Centre, Jaipur. Dengue Positive cases were characterized for serotyping. The identification of reference and clinical samples was determined using type specific primers & probe synthesized from IDT. ABI 7500 Fast Dx Real Time PCR (Pletier Block technology) & Type specific primer & probe were used to detect dengue serotypes. Detection of dengue virus was done on the basis of amplification of signals in the respective quencher dyes along with the detection of internal controls. Sequence of targeted dengue serotypes were as follows-[7]

DENV1_F CAATGGATGACAACAGAAGAYATG
DENV1_R TCCATCCATGGGTTTTCCTCTAT
DENV1_P TCAGTGTGGAATAGGGTTT FAM

DENV2_F GCAGAAACACAACATGGAACRATAGT
DENV2_R TGATGTAGCTGTCTCCRAATGG
DENV2_P TCAACATAGAAGCAGAACC VIC

DENV3_F ATGGAATGTGTGGGAGGTGG
DENV3_R GGCTTTCTATCCARTAGCCCATG
DENV3_P TATGGCTGAAACTCCGAG Tex as Red

DENV4_F GCAGATCTCTGGAAAAATGAACCA
DENV4_R GAGAATCTCTTCACCAACCCYTG
DENV4_P TCAATATGCTGAAACGC Cy5

Dengue viral RNA was extracted by using QIAamp Viral RNA mini kit (CAT No. 52906).[8]

MASTER MIX PREPARATION FOR SINGLE REACTION

ONE STEP MASTER MIX	12.5 µl
Enzyme	1.0 µl
DEN Primer Probe Mix	1.5 µl
Ext. RNA	10 µl
Total	**25 µl**

RESULTS

A total of 1192 dengue like illness cases who had less than <14 days of fever, blood samples were collected from various departments of Jaipur National University Institute for Medical Sciences & Research Centre for the diagnosis of Dengue viral infections. Out of 1192 cases 585 (49.07%) were Dengue were found.

Total 585 Dengue Positive cases were included in this study. Majority of the patients 161 males (27.52%) & 73(12.47%) females belonged to the 21-30 yrs of age group. Overall, 394(67.35%) patients were in age group of 11-30 yrs in this study.

Total 585 Dengue positive samples were further analysed for dengue serotype. out of 585 cases total 108 (18.46%) were DEN I, 163 (27.86%) DEN II, 218 (37.26%) DEN III and 74(12.64%) DEN IV were found. Along with this 24-mix serotype infection were also found such as 02 (0.34%) cases of DEN I & DEN II, 8(1.36%) cases were of DEN I & DEN III while 13(2.22%) cases of DEN II & DEN III and 01 (0.17%) Case of DEN III & DEN IV serotype was found. (Fig.1) All mix serotypes cases were found from Medicine ICU.

ANOVA test was performed to find out the co-relation with haematological parameters among dengue positive cases.

There was highly significant difference between the TPC of different types of DEN SEROTYPE as p of ANOVA = 0.000 < 0.05. As such we performed post hoc analysis and noted the following results:

1. TPC in Dengue Neg > DEN I, DEN II, DEN III, (DEN I & DEN III) and (DEN II & DEN III) mix infection
2. TPC in DEN I > DEN II
3. TPC in DEN I > DEN III
4. TPC in DEN I > DEN IV
5. TPC in DEN II < DEN III

We have received majority of the cases 349 (59.65%) from the Jaipur region followed by 56 (9.57%) from Karauli, 48 (8.20%) cases from Sawai Madhopur 42 (7.17%) from Dausa, 40 cases (6.83%) & 19 cases (3.20%) from Sikar & 18(3.07%) from were belong to Bharatpur.

Table 1: Seasonal trends of Dengue viral infection

Year	Dengue Positive	Dengue Negative	Total Cases
2018	189 (15.85%)	204 (17.11%)	393 (32.96%)
2019	240 (20.13%)	267 (22.40%)	507 (42.53%)
2020	156 (13.08%)	136 (11.40%)	292 (24.49%)
Total	**585 (49.0%)**	**607 (51%)**	**1192 (100%)**

Fig. 1: Circulating dengue serotypes in the study population of Rajasthan

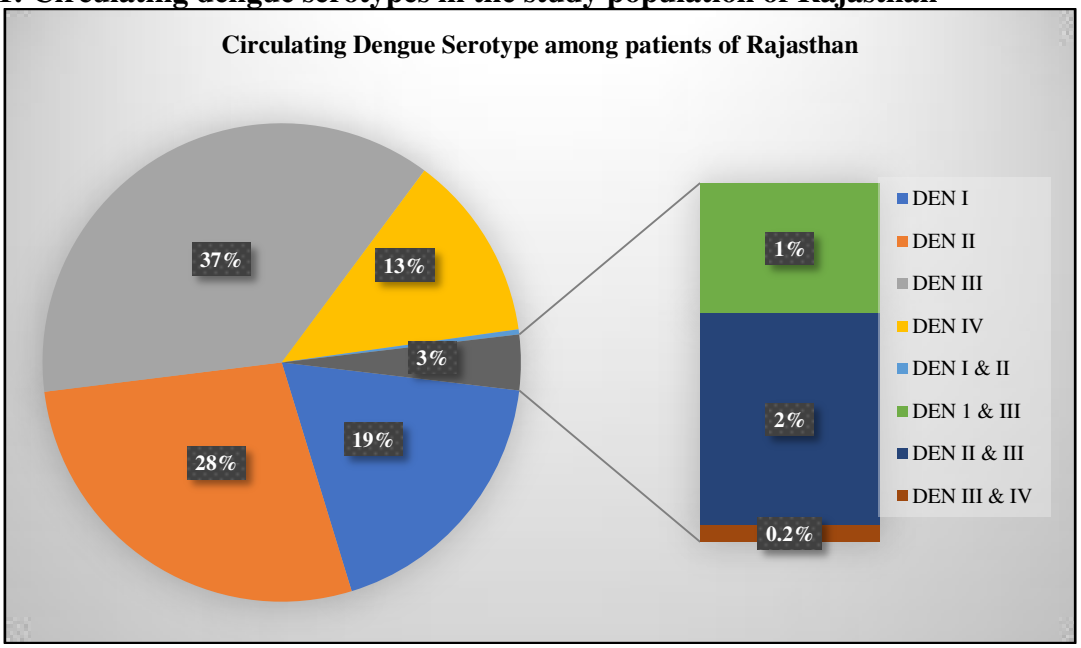

Table 2: Correlation of Dengue with hematological parameters (ANOVA)

ANOVA						
		Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
HB	**Between Groups**	.106	1	.106	.016	.899
	Within Groups	5519.270	841	6.563		
	Total	5519.376	842			
TPC	**Between Groups**	944992483088.204	1	944992483088.204	215.894	.000
	Within Groups	3681149828750.471	841	4377110379.014		
	Total	4626142311838.675	842			
RBC	**Between Groups**	1.072	1	1.072	1.187	.276
	Within Groups	759.801	841	.903		
	Total	760.873	842			
WBC	**Between Groups**	78.060	1	78.060	4.823	.028
	Within Groups	13611.281	841	16.185		
	Total	13689.341	842			
NEUTRO	**Between Groups**	3652.868	1	3652.868	11.504	.001
	Within Groups	267033.130	841	317.519		
	Total	270685.998	842			
LYMPHO	**Between Groups**	2978.033	1	2978.033	12.066	.001
	Within Groups	207576.699	841	246.821		
	Total	210554.732	842			

DISCUSSION

At present, laboratory diagnostic tools are very limited for diagnosing of Dengue viral infection is based on virus isolation, Molecular techniques targeting identification of virus genome offer rapid diagnosis and typing of DENV and are gradually replacing virus isolation as the new gold standard for DENV diagnosis in acute-phase serum samples.

In the present study Total 585 Dengue positive samples were serotyped for dengue. out of 585 cases total 108 (18.46%) were DEN I, 163 (27.86%) DEN II, 218 (37.26%) DEN III and 74(12.64%) DEN IV were found. along with this 24-mix serotype infection were also found such as 02 (0.34%) cases of DEN I & DEN II, 8(1.36%) cases were of DEN I & DEN III and 13(2.22%) cases of DEN II & DEN III and 01 (0.17%) Case of DEN III & DEN IV serotype was found. Thrombocytopenia, haemorrhagic manifestations and atypical presentations were found most commonly in DEN-3 followed by DEN-2 serotype. Coinfection with more than one serotype was observed in our study, with the most common coinfection pattern being DEN-2 and DEN-3 serotypes while in the study of Debunath F et al, 2020- DENV RNA was detected and quantified in 44, out of 79 patient samples by qRT-PCR. Forty-two DENV RNA positive samples were found to be DENV-1 and one was DENV-2 by the real-time HybProbe assay.[8]

Pramod Sidram Manthalkar et al Karnataka, 2019 in their study Out of 1000 serum sample test 462 serum samples were positive for dengue virus antigen or antibodies. Viral RNA was extracted from 119 samples positive for NS1 antigen by ELISA. Of the 119 samples tested for serotyping by RTPCR, 38 belonged to dengue serotype1 (DENV1), 46 were of dengue serotype 2 (DENV2) and 35 belonged to dengue serotype 3 (DENV3). A change in the earlier serotype 1 and 2 from 2011 to 2013 to the present serotype DENV2 and DENV3 was observed and constant presence of DENV2 in circulation was recorded.[9] In the study of Rubina paul et al, 2018 Out of her very acute phase dengue positive 50 samples, 35 sample (70%) were tested positive for dengue virus RNA in her study. NS1 positive samples. DENV-1 serotype was found in 20 samples, DENV-2 serotype was found in 1 sample and DENV-3 serotype was found in 14 samples. DENV-4 serotype was not found in any sample. Viral RNA was not detected in 15 samples.[10]

Meenakshi Kar et al; 2018, New Delhi were reported in 64 clinical isolates of DENV, mostly DENV-2. Twenty-five of these were further used for growth curve analysis in vitro, which showed a wide range of replication kinetics. The highest viral titers were associated with isolates from patients with dengue with warning signs and severe dengue cases.[11]while in the study of Parul D Shah et al; 2018 in Gujarat on the basis of RT-PCR, dengue infection was confirmed in 135 (55.78%) patients. DEN-3 was the most common serotype found in 71 (52.6%) patients, followed by DEN-2 serotype with 44 (32.6%) patients. Nearly 2.22% cases of DEN-2 and 2.96% cases of DEN-3 serotype were having dengue with warning signs. Severe dengue was found in 2.22% cases of DEN-2 and 5.18% cases of DEN-3 serotypes.[12]In the study of Shashi Sharma et al, 2017 at pune. 22 samples were found to be positive by single tube Dengue multiplex RT-PCR assay. Serotype DEN-2 was present in maximum numbers followed by DEN-3.[13]

CONCLUSION

Dengue remains to be an important health problem in India and across the globe. There is neither any specific treatment nor any effective vaccine for Dengue so far available in India. So, the proper diagnosis is the first requirement for the management of the disease. Secondary infection of Dengue greatly increases the risk for DHF and DSS if it is heterotypic. Both the conditions (DHF &DSS) are life threatening along. This makes the development of laboratory-based systems more important to provide an early warning of Dengue fever epidemics.

To conclude dengue serotyping was done by TaqMan probe as guided by CDC. The advantage with multiplex RTPCR was its ability to detect and serotype DENVs in samples with low viral load. Often during the acute phase of infection, the viral load comes down to low levels. Considering the rising number of Dengue fever cases in India and the neighboring countries, we suggest incorporation of an easy to perform and accurate molecular tool for serotyping the prevalent DENV strains, for which multiplex RTPCR may be considered as a fair alternative.

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