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Original Research Article

Serotyping of the Dengue Virus from a Clinical Sample by Using RT-PCR in A Tertiary Care Centre in Central India

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Abstract:

Background and Objective: The dengue virus is a positive-sense RNA virus that is enveloped and classified as a member of the genus Flavivirus within the family Flaviviridae. There are four different but closely related serotypes of the virus that cause dengue. First, second, third, and fourth DENVs. The objective of this study was to determine the serotypes of dengue virus in clinically suspected cases.

Material & Method: The present study was carried out from 2020 to 2021 in the department of microbiology at Mahatma Gandhi Memorial Medical College, Indore, Madhya Pradesh, and central India. Blood samples were received from suspected cases of dengue in the department of microbiology. All samples were tested for NS-1 antigen by the NS-1 ELISA kit. All positive samples by the NS 1 ELISA kit were subjected to detection of serotype by using real-time polymerase chain reaction.

Result: A total of 450 samples were tested, out of which 45 were NS1 ELISA positive and 42 were PCR positive. Of the 42 dengue RT-PCR-positive samples, DENV-2 was found in 37 samples, DENV-4 in 3, and a mixed serotype in 2.

Conclusion: The present study supports a better understanding of the different serotypes circulating in the study population and their association with the severity of the disease.

Keywords: DENV, NS 1 Antigen, Serotype, RT-PCR.

Introduction

Dengue virus is transmitted by female mosquitoes mainly of the species Aedes aegypti and, to a lesser extent by Ae. albopictus. Humans are mostly affected by the mosquito-borne disease dengue in tropical and subtropical areas of the world [1] Every year; about 390 million cases of dengue infection are recorded worldwide, of which 96 million results in clinical symptoms [2].

The dengue virus is spreading and transmitting around the world, and over 125 countries are located in dengue endemic zones, according to a World Health Organisation (WHO) research. Dengue fever is growing to such an extent that it is now native to about 100 nations worldwide, including those in Africa, America, the Eastern Mediterranean, South-East Asia, and the Western Pacific. The world's most severely impacted regions are those in the Americas, Southeast Asia, and Western Pacific [3]. The four distinct but closely related serotypes of the dengue virus are DENV-1, DENV-2, DENV-3, and DENV-4. The dengue virus is an enveloped, positive-sense RNA virus that causes the disease and is classified under the genus Flavivirus of the family Flaviviridae [4].

As of September 30, 2022, 63,280 cases of dengue were reported in India, according to the National Centre for Vector Borne Diseases Control. NCVBDC [5] The total seroprevalence of dengue infection in India is 48.7%.[6] Early diagnosis is crucial for spotting epidemics and outbreaks as well as for putting effective vector control measures in place. Various laboratory methods available to diagnose dengue infection are viral isolation, detection of RNA, antigen and antibody assays, and molecular methods. IgM and IgG antibodies are usually detected by ELISA using serologic testing. Numerous laboratories worldwide have investigated the detection of non-structural protein 1 (NS1) antigen in patients with primary and secondary infections during the acute phase of

their illness. All serotypes share NS1, a highly conserved glycoprotein that is generated in both secreted and cell membrane-associated forms [7,8,9,10].

Dengue virus serotyping helps in determining epidemic and hyperendemicity of dengue virus transmission. This study also provides information about epidemiological study.

Material Method

The present study was carried out from 2020 to 2021 in the department of microbiology at Mahatma Gandhi Memorial Medical College, Indore, Madhya Pradesh, central India. Blood samples were received from suspected cases of dengue in the department of microbiology.

Inclusion Criteria: All blood samples from suspected cases of dengue infection were included in this study.

Exclusion Criteria: hemolyzed blood samples and improper labeling.

All samples were tested for NS-1 antigen by the NS-1 ELISA kit (Med Source Ozone Biomedicals Pvt. Ltd.). All positive samples by the NS 1 ELISA kit were subjected to detection of serotype (Hi Media) by using real-time polymerase chain reaction (Bio-Rad Thermocycler PCR machine).

RNA extraction of Dengue virus

RNA extraction was performed using a commercial kit called TRUPCR, and the steps were followed according to the recommendations of the manufacturer.

Procedure: Pipette out 75 µl of proteinase K into a 15-ml falcon tube. Add 500 µl of sample into the micro centrifuge tube. Add 500 µl of buffer BAV1 (containing carrier RNA), Close the cap and mix by pulse vortexing for 15 seconds. Incubate at 56 degrees Celsius for 15 minutes. Add 600µl of molecular biology-grade ethanol (96–100%) to the sample, close the cap, and mix thoroughly by pulse vortexing for 15 sec.

Incubate the lysate with the ethanol for 5 minutes at room temperature, Load the lysate into the TRUPCR binding column and centrifuge at 10,000 rpm for 1 min. Add 600 µl of wash buffer 1 BAW1. Close the cap, centrifuge at 10,000 rpm for 1 minute, and discard the filtrate. Place the column in a clean collection tube and add 750µl of wash buffer 2 BAW2, centrifuge at 10,000 for 1 min, and discard the filtrate. Add 750 µl of molecular biology-grade ethanol (96–100%), centrifuge at 10000 rpm for 1 min, and discard the filtrate. Place the column in a clean collection tube and centrifuge at 14,000 rpm for 3 minutes to dry the membrane completely.

Real-time PCR (HiMedia Dengue serotyping probe PCR kit)

Master Mix Preparation: total volume of 25 µl contains RT buffer (DSO 221). 5µl, 10 X solution H (DSO222) 2.5µl, M-MuLV reverse transcriptase (DSO221) 1µl, DENV 1-4 primer probe mix (DS1186) 4µl, internal control primer probe mix (DS0498) 1µl, internal control DNA (DS1096) 1µl, molecular-grade water (ML065) 5.5 µl, template RNA/positive control/negative control 5 µl, Centrifuge the tube briefly at 6000 rpm for about 10 seconds.

Place the tubes in the real-time PCR machine and set the recommended PCR programme (mentioned below). Interpret the data from the amplification plot (observe the Ct values).

Recommended PCR programme Reverse transcription: 50°C for 15 minutes Initial denaturation at 95°C for 2 minutes, 30 seconds Denaturation, 95°C for 15 seconds, Annealing and Extension: 60°C for 1 minute (sampling) No. of cycles: 45 Channels: FAM/JOE/Texas Red/Cy5/Cy5.5, Hold: 4°C.

Table 1: Data Analysis the following conditions should be met for a valid diagnostic test:

Control	Detection channel				
	FAM (DENV-1)	JOE (DENV-2)	TexasRed (DENV-3)	Cy5 (DENV-4)	Cy5.5 (Internal control)
Positive control	+	+	+	+	+
Negative control	-	-	-	-	+

Table 2: Ct value and result interpretation

Ct value	Result
<40	Detected (+)
>40 or N/A	Not detected (-)

Result

Table 3: Total samples tested by NS1 ELISA and RT-PCR

Total suspected cases	NS1 ELISA positive	RT-PCR positive
450	45	42

A total of 450 samples were tested, out of which 45 were NS1 ELISA positive and 42 were RT-PCR positive.

Table 4: Percentage of various serotype detected

Serotype Detected	Number of Samples	Percentage %
DENV1	0	0
DENV2	37	88
DENV3	0	0
DENV4	3	7
mixed serotype	2	4.2
Total	42	100

Out of 42 dengue PCR-positive DENV- 2 were detected in 37 samples, DENV-4 in 3 samples, and a mixed serotype in 2 samples.

Table 5: age wise distribution of Dengue serotype

Age Group	Total Case	DENV1	DENV2	DENV3	DENV4	Co infection
1-15	4	0	4	0	0	0
16-30	22	0	19	0	2	1
31-45	15	0	13	0	1	1
46-60	1	0	1	0	0	0
P value		0.999				

In the age group 1–15-year DENV- 2 detected in 4 cases, in the age group 16–30-year DENV- 2 in 19 cases, DENV- 4 in 2 cases, coinfection in 1 case, in the age group 31–45-year DENV- 2 in 13 cases, DENV- 4 in 1 case, coinfection in 1 case. The P-value is not significant in different age groups for the dengue serotype.

Table 6: Gender distribution of Dengue serotype

Dengue serotype	Male	Female	Total
DEN1	0	0	0
DEN2	21	16	37
DEN3	0	0	0
DEN4	1	2	3
DEN2,3	0	1	1
DEN1,4	0	1	1
Total	22	20	42
P value	0.712		

Out of 42 RT-PCR -positive DEN 2 were detected in twenty males and sixteen females, DEN 4 in one male and two females, DEN 2 and 3 in one female, DEN 1 and 4 in one female. P value was not significant in the gender distribution of the dengue serotype.

Table 7: Month wise distribution of Dengue ELISA and PCR positivity

Months	Total Tested	Elisa Positive	Pcr Positive	Positivity	Serotype
January	21	2	2	9.5%	DEN2, DEN2,3
February	22	2	2	9%	DEN2
March	24	0	0	0%	
April	28	2	2	7%	DEN2
May	20	0	0	0%	
June	19	0	0	0%	
July	40	2	1	5%	DEN2
August	50	5	4	10%	DEN2, DEN4
September	61	6	5	9.8%	DEN2, DEN4, DEN1, 4
October	57	13	13	22%	DEN2, DEN4
November	58	7	7	12%	DEN2
December	50	6	6	12%	DEN2
Total	450	45	42		

A total of 450 samples were tested from January 2020 to December 2021; a total of 21 samples were tested in January, of which two were ELISA and RT-PCR positive. 22 samples were tested in February, of which 2 were ELISA and RT-PCR positive; 24 samples were tested in March; all were ELISA and RT-PCR negative; and 28 samples were tested in April, of which 2 were Elisa and RT-PCR positive. 20 samples were tested in May, of which all were ELISA and RT-PCR negative. 19 samples were tested in June; all are ELISA and RT-PCR negative. 40 samples were tested in July, of which 2 were ELISA positive and 1 was RT-PCR positive. 50 samples were tested in August, of which 5 were ELISA positive and 4 were RT-PCR positive. 61 samples were tested in September, of which 6 were ELISA positive and 5 were RT-PCR positive. 57 samples were tested in

October, of which 13 were ELISA and RT-PCR positive. 58 samples were tested in November, of which 7 were ELISA and RT-PCR positive. 50 samples were tested in December, of which six were ELISA and RT-PCR positive.

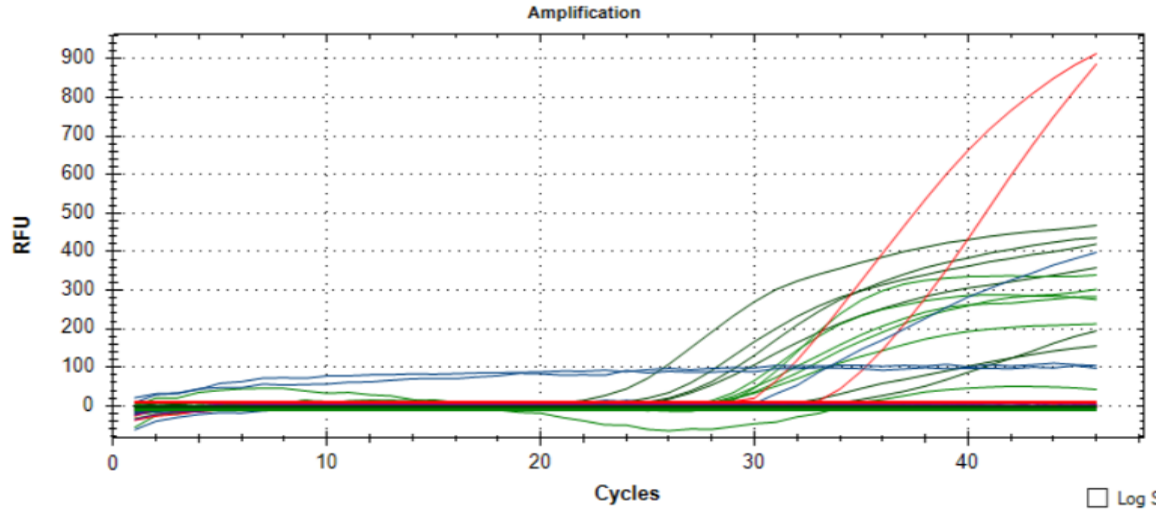

Figure 1:

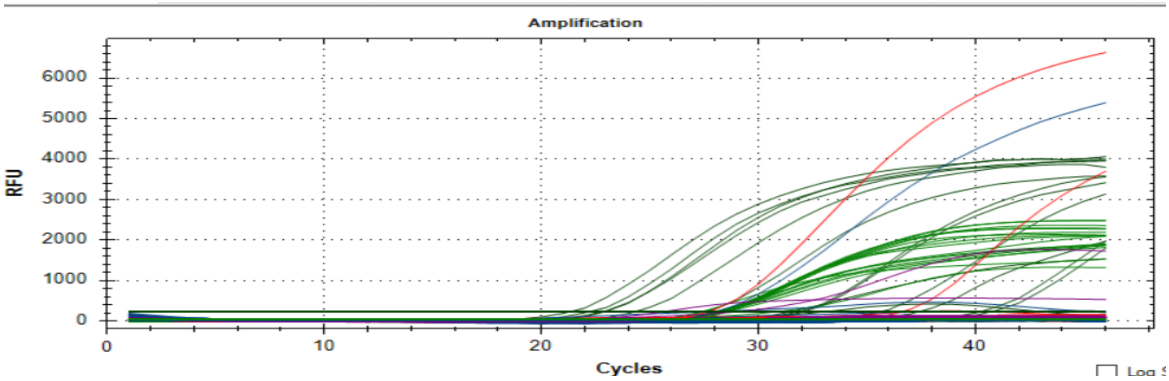

Figure 2:

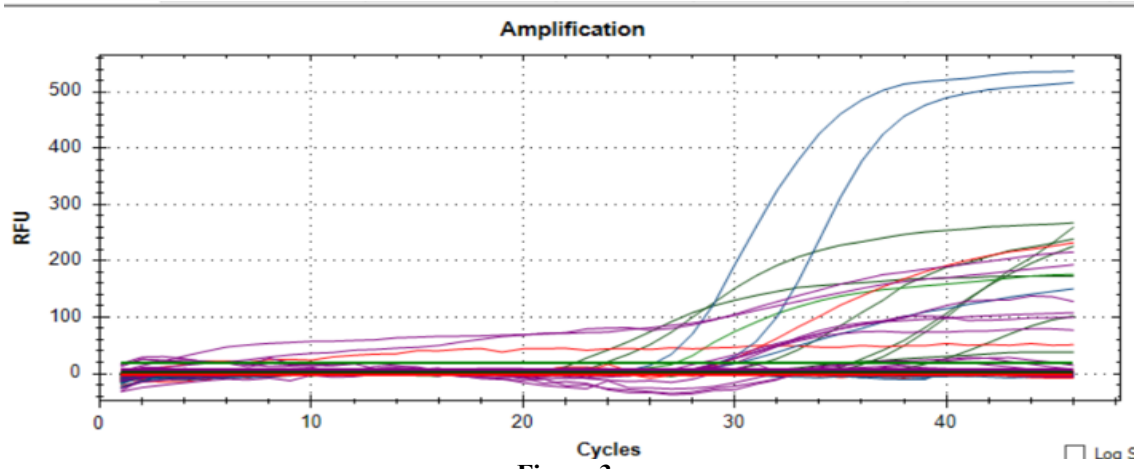

Figure 3:

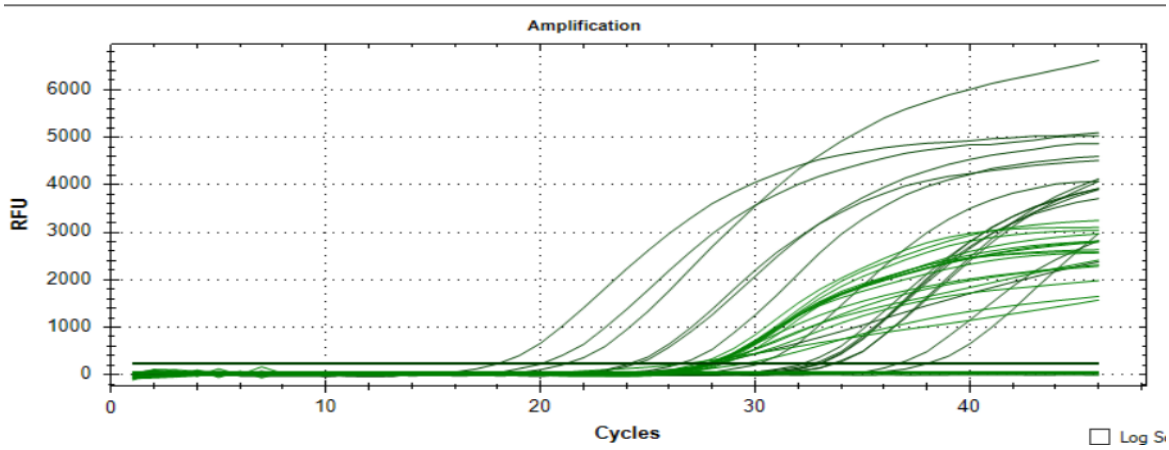

Figure 4:

Dengue virus serotypes: DENV 1- Purple colour, DENV 2 -Green colour, DENV 3 -Red colour and DENV4 -Blue colour

Discussion

A total of 450 samples were tested for dengue virus infection during the study period, among which 45 NS1 ELISAs were dengue-positive. Out of 45 positive samples, 42 were RT-PCR-positive. A total of 25 dengue samples were sent to the ICMR National Institute of Research in Tribal Health, Jabalpur, of which 20 were from our study and 5 were from routine samples of departments. Out of 20 samples in our study, 10 were NS1 positive and 10 were NS1 negative. Out of 10 NS1 positive samples, DENV 2 was detected in 9 samples, and DEN 2 and 3 were detected in one sample.

These 10 NS1 ELISA-positive samples were taken as an external control and tested here for serotype detection by PCR. The results were similar to our study, as 9 samples are Dengue serotype 2, and one sample is DEN 2,3. The NS1 positivity of dengue cases in the present study among clinically suspected fever cases was 10%. In the study conducted by Sathish J.V. et al. in 2018 at Chamarajanagar, Karnataka, the NS1 positivity was 10%, which is similar to our study. [11] In our study, the most common serotypes detected were DEN 2 (88%) in PCR-positive cases, followed by DEN 4 (7.14%) in 3 cases, mixed infection occurring in 2 cases (DENV 2 & 3) and (DENV 1 & 4) in our study.

Similar results were shown by Karma et al. in Sikkim, P.V Barde et al in 2015 and Dharitri Mahapatra et al. [12,13,14]. All four serotypes can be found in India because it is a developing nation. All four serotypes were identified in our research. DENV-2 was the most often found serotype, followed by DENV-4, and coinfection with DENV 1 and 4, and 2 and 3.

The increased urbanisation and ideal climatic conditions for mosquito growth in this area may be the cause of the hyperendemicity of DENV. Travel and an exponential rise in trade and tourism increase the likelihood of new DENV genotypes or serotypes being introduced through healthy viraemic persons, contributing to the development of a high transmission potential.

In this study, dengue infection was found frequently during the post monsoon period, i.e., September to November. It is because this season is favourable for the high breeding of vector mosquitoes.

As the monsoon season favours the breeding of Aedes mosquitoes, effective preventive and control measures need to be taken prior to the beginning of the monsoon to reduce the occurrence of dengue in the community.

Conclusion

The present study supports a better understanding of the different serotypes circulating in the study population and their association with the severity of the disease.

It clearly suggested that the molecular analysis along with serotyping of the dengue virus provide more reliable, accurate, and sensitive results when compared to the results obtained from the serological testing of the samples.

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