ORIGINAL RESEARCH ARTICLE

Dengue Serotype Prevalence and Laboratory Profile Correlation in a Tertiary Care Hospital in Gandhinagar

Bindu J Jadeja[1], Neeta Khokhar[2], Parul Patel[3], Neha Makwana[4], Gaurishanker Shrimali[5]

Authors' Affiliations: [1-5]GMERS Medical College, Gandhinagar, Gujarat
Corresponding Author: Dr. Neeta Khokhar, Email: neeta_khikhar@yahoo.com

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ABSTRACT

Background: Dengue fever and dengue haemorrhagic fever currently rank highly among the newly emerging infectious diseases and are the most important arboviral disease worldwide. Dengue virus can be distinguished by both serological and molecular methods. This study was aimed at analysing the prevalence and laboratory dynamics of the four dengue serotypes in tertiary care patients attending GMERS Medical College Gandhinagar.

Material & methods: This study was an observational retrospective study. A total 105 samples were tested for Dengue serotyping by RT-PCR.

Results: Among positive patients Dengue virus serotype-2 was the most common serotype 94 (89%) followed by DENV3 7(6%) and DENV4 2(2%). Co-infection with DENV 2/4 was 2(2%). A higher prevalence of dengue haemorrhagic fever was noted in serotype 2 compared to serotypes 3, 4, and coinfection. Thrombocytopenia was present in all serotypes of infection. There was a significant difference in the disturbance of liver function in DENV2, as compared to others serotype. Dengue serotype 2 was very common in rural areas, while dengue serotype 3 was seen in the urban Gandhinagar zone.

Discussion: Dengue is the most extensively spread mosquito-borne disease. As per previous studies most common prevalent and severity of serotype wasDENV2, however in our study we were able to identify DENV3, DENV4 and confection with serotypes (DENV2 & DENV4).

Keywords: Dengue serotype, multiplex RTPCR, Geographical distribution, thrombocytopenia

INTRODUCTION

The World Health Organization (WHO) estimates that approximately 50-100 million individuals are infected with dengue annually, and more than 2.5 billion individuals live at risk in more than 100 countries of dengue transmission [1]. Rapid unplanned urbanization and migration of population from rural to urban areas, lack of vector control, climatic changes, and poor sanitation facilities have contributed to fertile breeding areas for the dengue vector, Aedes aegypti [2]. WHO classification of dengue infection and grading of DHF are described in Annexure [3].

The dengue virus belongs to the family of Flaviviridae, and it consists of four closely related but antigenically distinct serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) [4,5].

Each one has its own unique manifestation depending on its interaction with the host's response. Infection with one serotype (primary infection) results in immunity to that serotype, but infection can occur with any of the remaining serotypes (secondary infection) [6]. Secondary DENV infection has been shown to be a significant risk factor for the development of severe disease, including dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS)[6,7].

Non-structural protein-1 (NS1) antigen capture assays are commonly used to identify dengue, results can be seen up to nine days after the onset of symptoms in primary infection. However, due to an anamnestic reaction, patients with secondary infection have NS-1 detectable for a significantly shorter time. Cross-reactivity with other flavivirus infections makes serological detection by immunoglobulin G or immunoglobulin M antibody capture assay difficult. In the acute phase of the disease (phase with viremia), molecular techniques, such as reverse transcription-polymerase chain reaction (RT-PCR), allow a same-day diagnosis of DENV and can also identify the serotype (even in patients with secondary infection). PCR-based techniques are sensitive, specific, fast, less complicated, and cheaper than virus isolation methods [8].

Previous research has examined the clinical and epidemiological characteristics of dengue infection. The goal of this study was to investigate the various dengue serotype along with their clinical and laboratory profiles in dengue patients at a tertiary care facility.

Aims and Objectives

The aims and objectives of the current study were to study the prevalence of dengue serotypes and severity of dengue infections among various serotype, to find association of gender and age groups with dengue serotypes, to find geographical distribution among different serotype and to correlate the serotype with the degree of thrombocytopenia, total leucocyte count, haematocrit, and disturbance of hepatic function in patient who attended GMERS Medical college and civil hospital Gandhinagar.

MATERIAL AND METHODS

Patient Enrolment

This study was an observational retrospective study. Participants of this study were those who presented to the OPD and Indoor patients at a tertiary-care hospital in GMERS Medical college and civil hospital Gandhinagar with signs and symptoms of fever, headache, and joint pain. The WHO standards were applied. The inclusion or exclusion of a dengue infection case and its classification as DF/DHF [3] follow. Clinically suspect patients provided samples, which were then forwarded to the laboratory department of the Civil Hospital, GMERS Gandhinagar. A dengue RT-PCR serotype was identified at the Gujarat biotechnology and research centre in Gandhinagar.

Laboratory evaluation

Blood samples were received from different OPDS & wards from suspected cases of dengue fever and tested for NS1 antigen by using 3RD generation ELISA (Oscar kit) at civil Hospital, Gandhinagar for duration of July2021 to September2021.Among all tested samples, positive samples were stored at -20˚c temperature and were sent to Gujarat Biotechnology Research Centre (GBRC) For further detection and identification of different serotype of dengue virus by using Multiplex RT PCR method.

Among the positive samples, laboratory parameters like total leukocyte count (TLC), platelet count, haematocrit count, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were also analysed for their association with a particular serotyping.

Data analysis

Data were analysed using the Microsoft excel software. Qualitative data were presented in the form of frequency and percentage; quantitative data were presented as mean ± standard deviation (SD). A p-value < 0.05 was considered as being statistically significant. Ethical approval was not required as it was an observational study.

RESULTS

A total of 105 samples were tested for isolation ofdifferent serotype of dengue virus by Multiplex RT PCR. DENV2 was found in 94 (89%) of the samples, followed by DENV3-7(6%), DENV4-2(2%) and DENV2/4-2(2%) respectively. DENV1 was not found in any samples.

Dengue classification according to World Health Organization guidelines published in 2011 based on signs and systems, as well as laboratory parameters (see ann)[3].

Table 1: Distribution of patients based on serotype and severity

Serotype	DF (%)	DHF (%)	Total (%)
DENV 1	-	-	0
DENV 2	49(46%)	45(43%)	94(89%)
DENV 3	5(4.2%)	2(1.8%)	7(6%)
DENV 4	1(1%)	1(1%)	2(2%)
DENV2/4	1(1%)	1(1%)	2(2%)
Total	56(52.2%)	49(47.8%)	105(100%)

DF-Dengue fever,DHF-Dengue haemorrhagic fever

Figure-1 Gender wise distribution on basis of serotype.

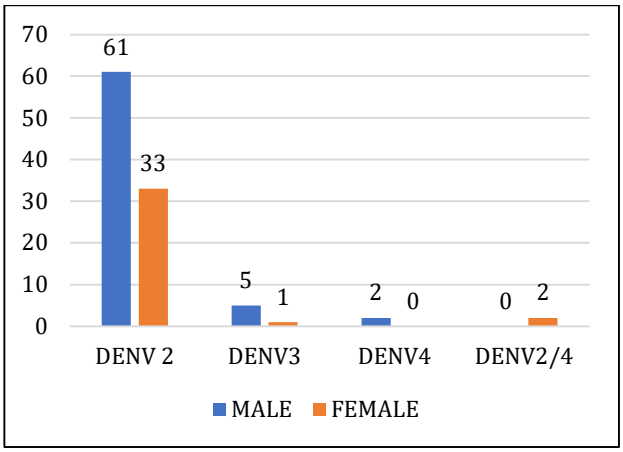

Figure -2 Age distribution of cases

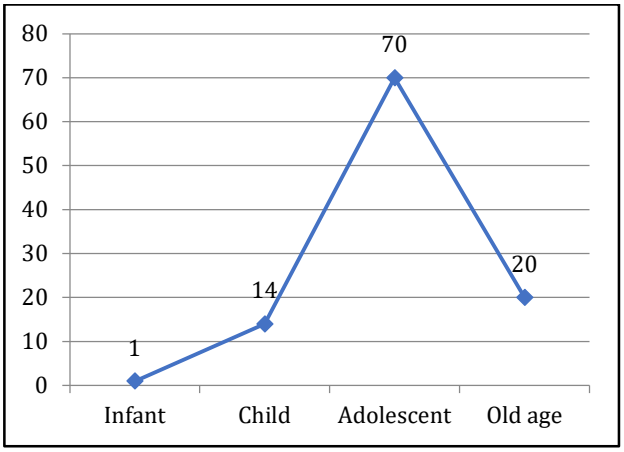

Figure-3: Geographical distribution of difference serotype.

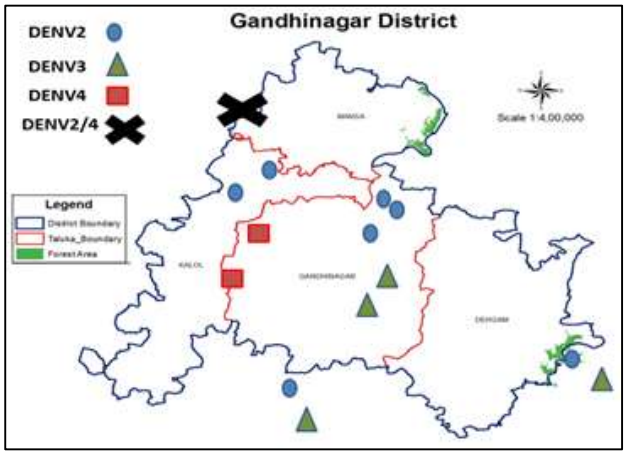

Figure 1 show association of male and female with different serotype. Figure 2 shows association of serotype with different age groups. Figure 3 shows distribution of serotype in Gandhinagar region.

Table 3 shows Association between Dengue serotypes 2 & 3 with laboratory profile of patients.

Table-2 Association between various laboratory Markers and Dengue Serotype 2 & 3

Laboratory Markers	Serotype	N	Mean	Std. Deviation	Mean Difference	95% CI of mean difference		t	p value
						Lower	Upper		
TLC	DENV 2	94	5090.96	2409.43	1601.24	322.18	2880.31	2.82	0.020*
	DENV 3	7	3489.71	1348.16					
platelet	DENV 2	94	132160.63	87629.90	5261.77	-62977.40	73500.94	0.15	0.879
	DENV 3	7	126898.86	90078.76					
Haematocrit	DENV 2	94	36.90	6.48	-1.81	-6.93	3.31	-0.70	0.485
	DENV 3	7	38.71	8.10					
SGPT	DENV 2	94	104.59	145.00	26.33	-83.25	135.90	0.48	0.635
	DENV 3	7	78.26	43.67					

*Statistically significant

Table-3Association between various laboratory Markers and Dengue Serotype 3 & 4

Laboratory Markers	Serotype	N	Mean	Std. Deviation	Mean Difference	95% CI of mean difference		p value
						Lower	Upper	
TLC	DENV 3	7	3489.71	1348.155	-1585.29	-4684.60	1514.03	0.046*
	DENV 4	2	5075.00	2793.072				
platelet	DENV 3	7	126898.86	90078.763	3148.86	-170252.78	176550.49	0.967
	DENV 4	2	123750.00	99348.503				
Haematocrit	DENV 3	7	38.7143	8.09762	0.21	-14.22	14.65	0.973
	DENV 4	2	38.5000	3.53553				
SGPT	DENV 3	7	78.2571	43.66508	-86.74	-163.39	-10.10	0.032
	DENV 4	2	165.0000	0.00000				

*Statistical significant

Table-4: Association between various laboratory Markers and Dengue Serotype 2 & 4

Laboratory Markers	Serotype	N	Mean	Std. Deviation	Mean Difference	95% CI of mean difference		p value
						Lower	Upper	
TLC	DENV 2	94	5090.96	2409.432	15.96	-3408.87	3440.79	0.99
	DENV 4	2	5075.00	2793.072				
platelet	DENV 2	94	132160.63	87629.899	8410.63	-116110.25	132931.51	0.89
	DENV 4	2	123750.00	99348.503				
Haematocrit	DENV 2	94	36.9037	6.47806	-1.60	-10.75	7.56	0.73
	DENV 4	2	38.5000	3.53553				
SGPT	DENV 2	94	104.5851	145.00321	-60.41	-265.05	144.22	0.56
	DENV 4	2	165.0000	0.00000				

Table-5 Association between all serotypes and laboratory profiles of patients using ONE WAY ANNOVA

		Sum of Squares	Mean Square	F	P value
TLC	Between Groups	20743800.704	6914600.235	6.246	.027
platelet	Between Groups	2074779520.165	691593173.388	.090	.965
Haematocrit	Between Groups	30.360	10.120	.237	.871
SGPT	Between Groups	16441.064	5480.355	.281	.839

Significant difference (p=0.02) between mean Total Leucocytes count (TLC) has been noted in Dengue serotype 2 & 3, while no significant difference has been found in other laboratory markers platelet count, Hematocrit value, SGPT viz.

Table-4 describe the association between serotype 2 & 4 with laboratory findings of patients. No significant difference has been found in any laboratory findings serotype 2 and serotype 4 patients.

Analysis of Variance (ANNOVA) shows only Total Leucocyte count of patients are significantly differ between all serotypes (serotype 2,3,4 and 2/4) while other laboratory finding of patients are not significantly differ between groups.

DISCUSSION

Dengue is the most extensively spread mosquito-borne disease. Its burden, prevalence, incidence, and distribution in Gujarat is necessary, as it shows an increasing trend

In our study, 105 patients with RT-PCR-confirmed dengue were studied. Out of those 105 cases, 95% were infected with dengue serotype-2 (DENV 2) and dengue serotype-3 (DENV 3) (Table 1). Maximum cases (89%) of dengue serotype-2, followed by 6% cases of DENV 3 and 2% cases of DENV4. This is in concordance with other studies from Delhi, Uttar Pradesh, and Mumbai [10-12]. In our study, two patients had co-infection with serotypes-2 and 4.

We found maximum cases of severe dengue in DENV 2 (89%) and DENV 3 (6%). However, this difference in distribution was statistically significant (p-value = 0.039).

Racherla et al. [13] in 2018, found DENV 2 as the predominant serotype in their study with higher severity of infection with DENV 3 and DENV 4.

This study also found that males are maximally affected than females in all dengue serotype but in co infection with serotype 2&4 female are more affected[figure-1]. Similarly, most of the reports found that a high male to female ratio was shown in most of the outbreaks in India [14,15]. The difference in gender may be due to social and cultural biasing with male predominance. Male partners are more exposed to various environmental factors in tropical and subtropical areas and are more prone to getting infection.

In our study, adolescents are affected more by all dengue serotype. Which is like study by Padhi S et al [16].

Figure 3 study also shows the geographical distribution of different serotypes in Gandhinagar district. DENV 2 is more common in outlying rural areas such as Kalol, Kolavda, Koba, and Uvarshad. DENV3 is found in sectors 10, 16, 21, and 23 of Gandhinagar urban zone. while DENV4 is seen in rural zone like Vavol and Sargansan. DENV 2/4 confection seen in Sadhav Rupal (rural zone). This shows rural Gandhinagar area more affected than urban zone with different serotype.

Mean total leucocyte was within normal limit in serotype 2, 4 and co-infection while lower level was seen in serotype 3[table 3,4,5] There was a statistically significant difference in total leucocyte counts between the serotypes 2 and 3 (p-value = 0.02) [table-2]. These results are in concordance with a study conducted by Wardhani et al. [17] in 2017.

Mean platelet count was lower in all serotype of dengue infection[table-2,3,4]. There was a statistically non-significant difference in the platelet counts between the different serotypes of infection (p-value = 0.965). [18].

There is no change Mean haematocrit value in different serotype. The mean SGPT levels was higher in serotype 2 with dengue viruses as compared to infection serotype 3,4 and co infection (Table 2,3,4) There was a statistically non-significant difference in the SGPT counts between the different serotypes of infection (p-value = 0.839) which is noticed with study with Ferede et al. [19]

As per table- 5 Analysis of Variance (ANNOVA) shows only Total Leucocyte count of patients are significantly differ between all serotypes (serotype 2,3,4 and 2/4) while other laboratory finding of patients are not significantly differ between groups.

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Annexure 1: WHO classification of dengue infections and grading of severity of DHF

DF/DHF	Grade	Signs and Symptoms	Laboratory
DF		Fever with two of the following: • Headache. • Retro-orbital pain. • Myalgia. • Arthralgia/bone pain. • Rash. • Haemorrhagic manifestations. • No evidence of plasma leakage.	• Leucopenia (wbc ≤5000 cells/mm3). • Thrombocytopenia (Platelet count < 150 000 cells/mm^3 • Rising haematocrit (5%-10%) • No evidence of plasma loss
DHF	I	Fever and haemorrhagic manifestation (positive tourniquet test) and evidence of plasma leakage	Thrombocytopenia <100 000 cells/mm^3; HCT rise ≥20%
DHF	II	As in Grade I plus spontaneous bleeding.	Thrombocytopenia <100 000 cells/mm^3; HCT rise ≥20%
DHF#	III	As in Grade I or II plus circulatory failure (weak pulse, narrow pulse pressure (≤20 mmHg), hypotension, restlessness)	Thrombocytopenia <100 000 cells/mm^3; HCT rise ≥20%
DHF#	IV	As in Grade III plus profound shock with undetectable BP and pulse	Thrombocytopenia <100 000 cells/mm^3; HCT rise ≥20%

Source: http://www.who.int/csr/resources/publications/dengue/Denguepublication/en/ #: DHF III and IV are DSS