

## A Cross Sectional Study of Prevalence of Various Dengue Serotypes Along with Hematological and Biochemical Profiles of Dengue Patients in Western Part of India

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### Abstract

**Introduction:** As per WHO, the 30 most highly endemic countries for Dengue in the world includes five countries as India, Indonesia, Myanmar, Sri Lanka and Thailand (December 2020). In India total 10172 cases were reported and 3 deaths were reported till 22 June 2022. In Dadara Nagar haveli, total 547 and 46 cases were reported during 2021 and till 22 June 2022 respectively.

**Objective:** The objectives of this study are 1) to find out prevalence of various Dengue serotypes of the given population 2) to compare hematological and biochemical parameters among the various dengue serotypes.

**Methods:** A hospital based cross sectional study was conducted in a tertiary care hospital at western part of India during January 2021 to December 2021. Those study participants who fulfilled the inclusion criteria were enrolled in the study. Hematological and biochemical parameter's tests were done. The statistical ANOVA test is applied to check significance of various parameters among serotypes.

**Results:** During the study period, a total of 100 dengue cases were included in the study. Mean age±SD of dengue patients was 27.15±10.82 years along with this 89% were in the age group of 11 to 40 years. Males were found to be 67 % and females were 33%. Maximum proportion of the patients belonged to DEN 2 serotype which is 53%, followed by DEN1 serotype, i.e., 23 %.

**Conclusion:** The study revealed that, dengue positivity was higher in male population than in females, DEN2 serotype was higher as compared to other serotype of dengue virus viz. DEN1, DEN3 and DEN4 as well as some of the patients were showing co-infection with serotypes-1 and 2 and serotypes-2 and 3. In dengue patient's hematological parameters like, PCV/Hematocrit was increased, while platelet counts decreased. Biochemical parameters like, ALT, AST, Serum Total Bilirubin, have found to be higher than normal range. Dengue Type 2 serotype is more severely causing reduction in platelet, leucocytes, RBC counts, and HB level as compared to other serotypes. This can be correlated with its antigenic structure that have more virulence power as compared to other serotype.

**Keywords:** Dengue, Serotype, Hematological parameters, Biochemical parameters

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## Background

There are four different serotypes of the dengue virus (DEN1, DEN2, DEN3 and DEN4) which causes dengue, it is caused by the virus named as an arbovirus (arthropod-borne viruses) which falls into *Flavivirus genus* of the family *Flaviviridae* [1,2]. Dengue presents with various clinical signs and symptoms and shows various clinical presentations, different stages of fever which varies from asymptomatic to an undifferentiated fever (viral syndrome) to the more severe types like severe dengue (SD) or Dengue hemorrhagic fever (DHF) [3]

Around 50-100 million individuals get dengue disease annually and more than 2.5 billion people around world are living at risk of Dengue transmission in more than 100 countries. Global warming, various climate changes, rapid urbanization, rural to urban migration, crowded areas with poor sanitation facilities causes favourable conditions for dengue vector, *Aedes Aegypti* [4].

According to WHO data, dengue cases increased over 8 times in last two decades, which are 505,430 positive dengue cases in the year 2000, to over 2.4 million in 2010, and 5.2 million in 2019. Total number of deaths from the year 2000 to 2015, it increased from 960 to 4032, in which mostly younger age group were affected. During years 2020 and 2021 total number of cases seems to be decreased, also the numbers of deaths were decreased. However, the data is not yet complete and COVID-19 pandemic might have affected case reporting among various countries. In 2020, several countries like in Bangladesh, Brazil, Cook Islands, Ecuador, India, Indonesia, Maldives, Mauritania, Mayotte (Fr), Nepal, Singapore, Sri Lanka, Sudan, Thailand, Timor-Leste and Yemen presented with reports of increases in the numbers of cases. During year 2021, in

countries like Brazil, India, Vietnam, the Philippines, Cook Islands, Colombia, Fiji, Kenya, Paraguay, Peru and Reunion islands, Dengue continued to be public health problem [5].

In India total 10172 cases were reported & 3 deaths were reported till 22 June 2022. In Dadara Nagar haveli, total 547 cases were reported during 2021, and 46 cases reported till 22 June 2022, while reported deaths were zero [6].

There are 4 serotypes of the dengue virus, which are called as DENV 1, DENV 2, DENV 3, and DENV 4. The Dengue infection can happen by any of the four serotypes, it also provides lifelong immunity to the same serotypes [7].

## Methodology

### Material and Method

**Study design:** The study was conducted in a tertiary care hospital in western part of India during January 2021 to December 2021. Study type is cross sectional and sampling technique is purposive sampling. All the patients who walked in with symptoms of Dengue and who were ready to be the part of the study were included. Those who denied or were seriously debilitated were excluded from the study. Patients consent was taken, and study was conducted with prior permission of the institute and ethical committee.

**Data analysis:** Data was collected, compiled and analyzed using Microsoft excel and SPSS 16 software. Descriptive statistics such as mean  $\pm$  SD were done for all the continuous variables. Statistical significance between various parameters were checked using t test and ANOVA test. Statistical significance level was set at  $\alpha = 0.05$

**Sample size and Selection procedure:** From 7266 Blood samples were collected

from suspected dengue cases during January 2021 to December 2021 attending outpatient and medical wards of a tertiary care hospital. Various tests like various serological, hematological and biochemistry tests were performed on these patients.

All the samples collected from suspected dengue cases were tested for Dengue NS1 Ag ELISA (Panbio Dengue Early ELISA (Alere, Brisbane, Australia), according to manufacturer's instructions.

Out of these, 802 patients tested positive for dengue NS1 Ag ELISA in the Microbiology lab that were also include patients from Maharashtra and Gujrat also. Total 547 dengue positive patients were belonging to DNH. The dengue NS-1 Ag positive serum samples were stored at -80 °c. In March 2022 RT PCR was done for dengue serotyping of 100 dengue NS1 Ag ELISA positive sample of Dadra Nagar

haveli only. For molecular serotyping of DENV the Nucleic acid extraction was performed by using the QI Aamp Viral RNA Mini Kit (Qiagen; Valencia, CA). All extractions were carried out as per the manufacturer's recommendations and guidelines. Extraction was performed by using 140 µL of patient serum, eluted into 60 µL of buffer AVE on QIA cube (Qiagen; Valencia, CA). The DENV multiplex RT-PCR was performed using the Superscript III Platinum One-Step qRT-PCR kit (Invitrogen; Carlsbad, CA) as per the CDC protocols (2013).

### Objectives

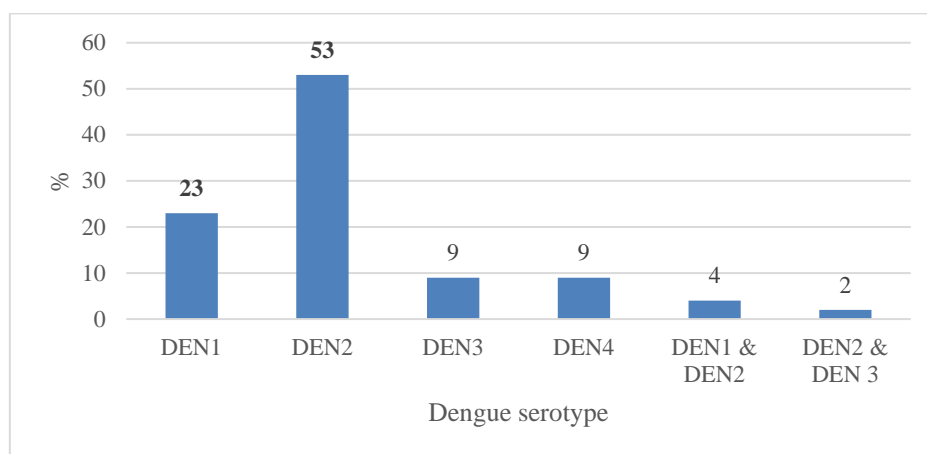
- 1) To find out prevalence of various dengue serotypes of the given population,
- 2) To compare hematological and biochemical parameters among the various dengue serotypes.

### Results

**Table 1: Demographic distribution of dengue patients along with serotype (n=100)**

Dengue serotype		DE N1	DEN 2	DEN 3	DEN 4	DEN1 & DEN2	DEN2 & DEN3	Total
Gender	Male	16	39	4	4	2	2	67
	Female	7	14	5	5	2	0	33
Age group (years)	0-10	1	1	1	0	0	0	3
	11to 20	7	11	2	0	1	1	22
	21-30	6	29	2	3	1	1	42
	31-40	8	9	3	4	1	0	25
	41-50	0	2	1	1	1	0	5
	51-60	1	0	0	1	0	0	2
	>60	0	1	0	0	0	0	1
Total		23	53	9	9	4	2	100

Out of 100 positive samples, males were found to be 67 % and females were 33%. Maximum numbers of patients were seen in the age group of 11 to 40 years, which is 89 %.



**Figure 1: Distribution of dengue serotype (n=100)**

Maximum proportion of the patients belong to DEN 2 serotype, which is 53%, followed by DEN1 serotype, i.e., 23%.

**Table 2: ANOVA for Hematological parameters among various serotype of dengue (n=94)**

Type	DEN1		DEN2		DEN3		DEN4		F	p
	Mean, n=23	SD	Mean, n=53	SD	Mean, n=9	SD	Mean, n=9	SD		
Age (years)	26.13	10.11	27.15	11.00	28.36	12.71	27.45	11.90	2.267	0.086
HB	13.14	1.84	13.12	1.83	13.35	1.67	13.22	1.80	0.738	0.532
TLC	5.45	3.22	5.36	3.21	5.65	3.58	5.44	3.24	3.659	0.015
RBC	4.64	0.60	4.63	0.59	4.73	0.53	4.68	0.58	0.833	0.479
PCV /Hematocrit	56.04	5.27	60.97	5.23	59.64	4.75	65.24	5.13	0.485	0.039
PLT	1.45	0.84	0.98	0.79	1.12	0.70	1.46	0.82	0.807	0.014
POLYS	65.66	12.77	65.54	12.54	65.69	12.40	64.49	12.57	0.428	0.733
LYMPHO	30.21	13.23	30.32	13.02	29.57	11.75	31.41	13.46	0.42	0.739
EOSIN	2.09	1.03	2.11	1.02	2.15	1.13	2.11	1.04	0.467	0.706
MONO	2.48	1.44	2.48	1.43	2.63	1.52	2.57	1.46	0.593	0.621
BASO	0.02	0.20	0.02	0.20	0.04	0.27	0.03	0.23	1.03	0.383
ESR	14.98	9.28	14.78	9.29	14.38	8.56	14.83	8.70	0.323	0.809

**Note:** HB: hemoglobin (gm%); TLC: total leucocyte count (1000\_per\_cumm); RBC: Red blood cells (mill\_per\_cumm); PCV: Packed Cell Volume or hematocrit (%); PLT: platelet (Lac\_per\_cumm); Polys: Polymorphs (%); LYMPHO: Lymphocytes (%); EOSIN: Eosinophils (%); MONO: monocytes (%); BASO: Basophil (%); ESR: erythrocyte sedimentation rate (millimeters in one hour (mm in 1 hr)).

All the abbreviation of parameters and their measurement units were same throughout this article.

The above table shows statistically significant difference of hematological parameters among various serotype of dengue. All the hematological parameters having average values/counts

except, PCV/Hematocrit and platelet counts. Further, PCV/Hematocrit was increased while platelet counts decreased in various dengue serotypes patients. Significant difference was found in mean values of TLC, PCV/Hematocrit and platelet counts among serotypes ( $p < 0.05$ ).

**Table 3: ANOVA for biochemical parameters among various serotype of dengue (n=94)**

Type	DEN1		DEN2		DEN3		DEN4		F	p
	Mean, n=23	SD	Mean, n=53	SD	Mean, n=9	SD	Mean, n=9	SD		
CRP - Quantitative	2.22	2.66	2.07	2.48	1.91	2.45	1.99	2.31	3.485	0.019
Serum Total Bilirubin	1.90	1.44	2.42	1.43	2.23	1.87	2.62	1.61	2.839	0.042
SGPT (ALT)	79.89	48.91	76.98	48.50	80.45	39.90	82.12	47.85	1.241	0.3
Serum Alkaline Phosphatase	97.29	60.57	96.89	60.11	90.58	57.92	97.03	60.70	0.647	0.587
SGOT (AST)	64.41	87.56	68.83	95.40	55.55	46.92	60.84	83.29	0.509	0.677
Albumin	3.69	0.51	3.68	0.50	3.66	0.54	3.68	0.53	0.927	0.431
Serum Sodium	137.86	3.38	137.75	3.40	138.36	3.02	138.14	3.02	0.474	0.701
Serum Potassium	4.00	0.52	3.99	0.52	4.00	0.60	4.01	0.57	0.806	0.494
Serum Chloride	98.99	10.75	98.89	10.65	98.02	13.86	98.77	11.98	0.321	0.81
Serum Creatinine	0.86	0.23	0.87	0.18	0.87	0.21	0.86	0.28	0.26	0.843
BUN	11.32	6.85	11.37	6.79	12.58	7.81	11.80	7.17	2.929	0.038
BSR	88.41	14.31	88.45	14.38	88.27	14.24	87.93	14.48	0.676	0.569

**Note:** CRP:C-reactive protein; Serum Total Bilirubin (mg/dl); SGPT (ALT): Serum Glutamic Pyruvic Transaminase (alanine aminotransferase) (IU/L); Serum Alkaline Phosphatase (IU/L); SGOT (AST):Serum glutamic-oxaloacetic transaminase (aspartate aminotransferase) (IU/L); ALBUMIN (gm/dl); Serum Sodium (milliequivalents per liter: mEq/L); Serum Potassium (mEq/L); Serum Chloride (mEq/L); Serum Creatinine (milligrams per deciliter: mg/dl); BUN: blood urea nitrogen (mg/dl); BSR: blood sugar random (mg/dl).

The above table shows statistically significant difference of biochemical parameters among various serotype of dengue. In this study, ALT, AST, Serum Total Bilirubin have found to be higher than normal range.

## Discussion

In our study, 100 patients with RT-PCR-confirmed dengue were studied. Out of those 100 cases, 53% were infected with dengue serotype-2 (DENV 2) followed by 23% DEN1 serotype. 4% and 2% cases were infected with DENV 1 & DENV 2

and DEN2 & DEN3 respectively i.e., six patients had concurrent infection with two serotypes of the dengue virus. Four patients had co-infection with serotypes-1 and 2, and two patients had co-infection with serotypes-2 and 3 (Table 1). In study

done by Gupta A, Rijhwani P *et al*, four patients had concurrent infection with two serotypes of the dengue virus. Three patients had co-infection with serotypes-1 and 3, and one patient had co-infection with serotypes-2 and 3. [9].

In our study, males were found to be 67 % and females were 33% with maximum numbers of patients i.e., 89 % were seen in the age group of 11 to 40 years (Table 1). A study done by Ferede *et al*. showed similar findings as positive cases of dengue were higher in male population (76.5%) than in females (23.5%). Dengue patients were higher in >15 years of age group (84.3%)[8].

Out of 100 dengue patients, 53% belonged to DEN 2 serotype, followed by 23% DEN1 serotype (Figure 1). A study done by Aviral Gupta *et al*. done at Northwestern India also showed that dengue virus serotype-2 (DENV 2) was the most common serotype i.e., 34% [9].

The same kind of results were also found in the study done in Delhi, Uttar Pradesh, and Mumbai [10,11,12].

Significant difference was found out between Total Leucocyte count ( $p=0.015$ ) in dengue serotype 2 as compared to other serotype and in the mean platelet count amongst the groups ( $p= 0.014$ ) (Table 2), while a study done by Aviral *et al*. showed no significant difference in the mean leucocyte count ( $p$ -value 0.575) and in the mean platelet count ( $p$ -value=0.190) among the groups respectively [9].

In this study, Haematocrit count was increased than normal, while platelets were found to be decreased in various dengue serotypes.

In a study done by Kao *et al.*, Ageep *et al.*, showed that there was a 20% increase in hematocrit from the patient's baseline, which is also linked with thrombocytopenia ( $< 100 \times 10^9/L$ ) [13,14]. As per table 3, ALT, AST, Serum Total

Bilirubin is higher than normal range. A study done by Kuo *et al* showed Raised

AST levels in 63%-97% of patients, increased ALT levels in 45%-96% of patients. [15]. In a study done by Prakash *et al*. showed that average levels of AST ranged from 93.3 U/ L[17] to 174 U/L [16]. Other studies showed that ALT ranged from 86 U/L [16,17], Clinical jaundice was found in 1.7%-17% [16,18,19] and hyperbilirubinemia was also found to be as high as 48% [20].

Significant difference in mean values of biochemical parameters like CRP, total bilirubin and BUN among various dengue serotypes ( $p<0.05$ ).

In this study there is no significant difference in mean values of ALT, AST and serum Albumin, while a study done by Aviral *et al*. shows a statistically significant difference in the mean values of ALT, AST and serum albumin levels ( $p$ -value  $<0.001$ ).

In this study BUN levels were found to fall within normal range in all Dengue serotypes (Table 3), the similar type of results was seen in study done by Aviral *et al*. [9].

## Conclusion

The study revealed that, dengue positivity was higher in male population than in females, DEN2 serotype was higher as compare to other serotype of dengue virus viz. DEN1, DEN3 and DEN4 as well as some of the patients had co-infection with serotypes-1 and 2 and serotypes-2 and 3. In dengue patient's hematological parameters like, PCV/Hematocrit was increased while platelet counts decreased. Biochemical parameters like, ALT, AST, Serum Total Bilirubin, have found to be higher than normal range. Dengue Type 2 serotype is more severely causing reduction in platelet, leucocytes, RBC counts, and HB level as compared to other serotypes. This can be correlated with its antigenic structure that have more virulence power as compared to other serotype.

## Limitation of Study

Sequencing is missing that confirmingly shows gene responsible for virulence effect of various serotype of Dengue virus.

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