



Dengue infection profile in Gujarat, West India: A recent report

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ABSTRACT

Dengue fever (DF) is a mosquito-borne viral disease occurring in tropical and subtropical areas including India. It is manifested by symptoms like fever, headache, rashes, muscle, and joint pains. In our country, Gujarat is one of the highest infected states by this disease due to mosquito bites. Blood samples from such cases were collected and assessed for blood counts, serum transaminases, and blood smears from only positive patients using standard protocols. Real-time PCR (RT-PCR) technique was also employed for virus detection. Of 1020 referral cases, from Ahmedabad, Vadodara, Jamnagar, and Anand districts, 184 cases were detected positive (18.03%) from October 2016 to December 2019 (39 months) being highest in 2019. Males had a high frequency (52%) of infection when compared to females (48%), affecting young age groups ranging from moderate to higher level. Furthermore, Ahmedabad district (57%) had a high percentage of infection, compared to others. Marked alterations were also observed with respect to serum glutamate-pyruvate transaminase and serum glutamic oxaloacetic transaminase, platelets, lymphocytes, and WBC counts in positive cases of 0–20, 21–40, and 40–50 year age groups. Reduced platelet clumping and severe thrombocytopenia (59%) in these cases are early diagnostic markers of dengue, supporting RT-PCR method. Thus from our study, we conclude that dengue infection is caused by various factors such as sex, climate, population, and environment. This outbreak is hence controlled by following health measures strictly, imposed by health departments in Gujarat, Western India.

1. INTRODUCTION

Dengue is a severe, flu-like illness that affects infants, young children, and adults but sometimes cause deaths. This fever is caused by the *Aedes aegypti* mosquito bite and is manifested by four dengue viruses occurring in tropical and subtropical areas of the world including India. Its severity ranges from dengue fever (DF) and dengue hemorrhages fever (DHF) to dengue shock syndrome (DSS) in India and around the globe [1–5]. Each one has its own unique manifestation depending on its interaction with the host's response [4–8].

It is a major global public health concern, as more than 2.5 billion people are estimated with this infection [3,9] and more than 50 × 10⁶ new cases are being projected annually [4,10]. Recently, the National Vector Borne Disease Control Program projected that

dengue-infected cases are topped in Gujarat in comparison to Karnataka [11], although other states and union territories showed marginal increments till 2019 and then exhibited a decline in dengue cases due to probable increased controlling measures and better sanitization facilities in India.

Outbreaks and deaths due to this have been recorded in the last decade from the Northern states of Haryana, Punjab, and Uttar Pradesh, Southern states of Andhra Pradesh, Tamil Nadu, Kerala, and Karnataka, Western parts of Gujarat and Rajasthan, as well as the Eastern states like West Bengal [3,4]. In Gujarat, as percentage of dengue cases increases continuously; this work has been undertaken to investigate this outbreak in four districts, namely Ahmedabad, Baroda, Jamnagar, and Anand. Since more cases get referred to our center of Neuberg-Supratech Reference Laboratory and Research Institute, Ahmedabad, from 2016 to 2019 (39 months) their frequency was calculated to assess controlling measures adopted by respective authorities, and other causative factors in Gujarat, Western India.

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2. MATERIALS AND METHODS

2.1. Patients' Selection

Patients (1020) suspected for DF based on clinical reports and exposed to mosquito bites from four district centers were referred to our main center, i.e., Neuberg-Supratech Reference Laboratory and Research Institute, Ahmedabad. These referral cases were divided into four age groups: 0–20, 21–40, 41–50, and 51 and above from October 2016 to December 2019 (39 months). Each patient was asked to fill out a concern form, followed by blood collection on priority basis. Then, analysis was carried out after approval of our ethical committee for investigation (GU/HEC-001-2015) Ahmedabad, in 2015.

2.2. Hematological and Biochemical Profiles

Complete blood cell count was carried out using cell counter (Siemen's Advair 2010, Germany). Serum transaminases of serum glutamic oxaloacetic transaminase (SGOT)/aspartate transaminase and serum glutamate-pyruvate transaminase (SGPT)/alanine transaminase were estimated using a kit (Dimension EXL, Germany), which was expressed as U/l. Blood smear for positive cases was prepared using Leishman stain and the peripheral blood film was observed under the microscope for morphology of blood cell types like lymphocytes, neutrophils, RBC, and platelets under the light microscope from lower to higher magnification [12]. The report on these cell types was assessed by pathologists in our laboratory.

2.3. Dengue Viral Detection

The real-time PCR (RT-PCR) assay used in this study for the detection of the dengue virus was TaqMan's primer and probe mixer by in-house method. The assay allowed for a qualitative detection of dengue virus-specific RNA, with reverse-transcription of the RNA into complementary DNA, followed by PCR for the amplification of specific target sequences. The probes utilized were specific for DENV RNA labeled with the fluorophore FAMTM and specific for the internal control labeled with the fluorophore VICTM. The in-house protocol used QuantStudio5 instrument for Ab detection. The data obtained were entered into the Microsoft Excel spread sheet and descriptive statistics were carried out to obtain C analysis of variance. *p*-value less than 0.05 was considered significant.

3. RESULTS

Of 1,020 referral cases, 184 cases were positive for DF (184/1,020 = 18.03%). During 39 months of our study, Jan–Dec 2019 had the highest frequency of cases (54/259 = 20.84%), followed by Jan–Dec 2018 (63/326 = 19.32%) (Table 1). The young age group (0–20; 21–40 years) possessed a higher percentage of cases in both sexes. However, males (52%) had a high percentage of affected cases in comparison to females (48%) in our report (Table 2).

3.1. Biochemical and Hematological Profile

The levels of SGPT and SGOT had a higher percentage than normal levels in all age groups. However, this trend had declined

Table 1: Year-wise prevalence of dengue cases referred in Oct 2016–Dec 2019 (39 months).

Year	Dengue		
	Total	Positive	Percentage
Oct–Dec 2016	150	24	16.00
Jan–Dec 2017	285	43	15.08
Jan–Dec 2018	326	63	19.32
Jan–Dec 2019	259	54	20.84
Total	1,020	184	18.03

with increased age (Table 3). The platelets count was reduced in all positive cases, compared to negative cases. It was significant ($p < 0.0170$) in the 21–40 years age group. Similarly, WBC counts significantly ($p > 0.0004$, $p > 0.0003$, and $p > 0.0262$) reduced in three young age groups of positive cases, respectively. The same trend was found with respect to lymphocytes ($p < 0.0001$ and $p < 0.0210$) in all age groups (Table 4). Among the four districts of Gujarat, Ahmedabad topped (57%) the cases, followed by Vadodara (17%), Jamnagar (16%), and Anand (10%) respectively (Fig. 1).

3.2. Blood Cell Morphology

Blood films were done for all positive cases (184) and cell abnormalities were observed by the pathologists. RBC had nucleated (27%; 50/184) and hypochromic and neutrophil exhibited fragmentation (42/184; 23%), whereas lymphocytes became atypical, as shown in Figure 2 (68/184; 37%). Platelets in some peripheral blood films contained no agglutination/clumping with thrombocytopenia (109/184; 59%), which are markers for dengue infection (Fig. 3).

4. DISCUSSION

Dengue is the most extensively spread mosquito-borne disease, endemic in more than 100 countries around the globe. Its burden, prevalence, incidence, and distribution in Gujarat is necessary, as it shows an increasing trend [5,11]. The study carried out by us depicted a sharp rise in positive samples from year 2017 to 2019, in support of earlier data presented [11,13,14]. The frequencies of infected cases also were higher in months corresponding to monsoon season [3,4,15].

In general, all age groups are affected by DF in India [12,16,17]. Younger age groups are more commonly infected by DF in support of our data where the 21–40 years age group is has both sexes infected. Dinkar and Singh [15] reported that the 20–30 age group is more infected as found during the epidemic in Delhi and other states like Chandigarh, Haryana, Maharashtra, Punjab, and UP. Predominantly children and adults of these age groups are active, who were out of the house most of the time either playing or at work [18–21]. But on the other hand, few reports recorded a higher rate of cases in the 5–12 age group due to lack of immunity [2].

This study also found that males are maximally affected by DF than females. Similarly, most of the reports found that a high male to female ratio was shown in most of the outbreaks in India

Table 2: Age and gender-wise percentage (%) of positive dengue cases (184) in the study (Oct 2016–Dec 2019).

Age group	Male		Female		Total patients	
	Positive	Percentage	Positive	Percentage	Positive	Percentage
0–20	36	38	26	29	62	33.69
21–40	40	42	35	40	75	40.76
41–50	04	4.2	14	16	18	09.78
51 above	16	16	13	15	29	15.76
Total	96	52	88	48	184	18.03

Table 3. Percentage (%) of serum transaminase level in dengue cases (184) (Oct 2016–Dec 2019) in the four age groups.

Age group	SGPT (100%) (U/l)	SGOT (100%) (U/l)
0–20	60.33 (165.28%)	57.92 (156.54%)
21–40	57.88 (158.57%)	72.02 (194.64%)
41–50	72.01 (197.28%)	67.02 (181.13%)
51 above	51.14 (140.93%)	40.66 (109.89%)

Normal range – SGPT: 14–59 U/l; SGOT: 0–37 U/l.

Table 4: Blood cell count in dengue-positive cases versus negative cases (Oct 2016–Dec 2019) in our study.

Blood cell type	Case type	Age groups in years			
		0–20	21–40	41–50	51 above
Platelets count (150,000–410,000)/ μl	Negative	257,173 ± 118,405	226,385 ± 98,188	218,157 ± 83,346	208,445 ± 91,767
	Positive	217,705 ± 96,935	185,033 ± 68,727*	191,000 ± 48,862	174,718 ± 55,447
	<i>p</i> value	0.1723	0.0170	0.3112	0.1439
RBC (3.8–4.8 Million/Cumm)	Negative	4.85 ± 0.85	4.62 ± 0.50	5.34 ± 5.62	4.58 ± 1.41
	Positive	5.01 ± 0.51	4.49 ± 0.69	4.52 ± 0.69	4.31 ± 0.70
	<i>p</i> value	0.4158	0.2164	0.6493	0.4412
WBC (4,000– 10,000)/μl	Negative	7,599 ± 3,780	6,100 ± 2,875	5,985 ± 2,611	6,571 ± 3,268
	Positive	4,405 ± 2,171*	4,214 ± 2,052**	4,012 ± 1,594***	5,501 ± 2,133
	<i>p</i> value	0.0004	0.0003	0.0262	0.1947
Lymphocytes count (1,000–3,000)/ μl	Negative	1,912 ± 1,094	1,291 ± 783	1,147 ± 693	1,377 ± 1,259
	Positive	929 ± 411@	768 ± 347@	613 ± 267	913 ± 657
	<i>p</i> value	0.0001	0.0001	0.0210	0.1410
Hb (12.0–15.0 G%)	Negative	13 ± 2.19	13.1 ± 1.76	12.6 ± 2.24	13.44 ± 8.52
	Positive	13.81 ± 1.58	12.89 ± 2.12	12.88 ± 0.85	12.40 ± 1.86
	<i>p</i> value	0.1213	0.5494	0.7030	0.6181

Values are Mean ± SE; Figures in parentheses indicate normal range.

[3,4,15,22,23]. 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 sub-tropical areas and are more prone to getting infection [3,24,25].

A regional study of Gujarat screened in the four districts showed Ahmedabad had a higher frequency of the dengue virus, compared to Varodara, Jamnagar, and Anand. This could be due to migration of population for better survival, poor sanitization facilities, and seasonal variants for the vectors, *A. aegypti* and *Ae. albopictus* and environmental conditions [1,2,26,27]. The data were supported by drastic increase in serum aminases in our study. This increase in SGPT and SGOT levels is correlated to liver dysfunction due to DF. Other biochemical profiles like serum protein, albumin, and

urea levels also altered dengue infection to separate Chikungunya [6] in addition to higher hematocrit, tachycardia, cough, and fever in dengue cases to boost our results [4].

Hematological profile exhibited significantly reduced platelet count, followed by WBC in dengue patients in collaboration with the data of others [4,6,26]. Increased thrombocytopenia and lack of platelet clumping in our present study also challenged the condition of dengue. Decrease in lymphocytes and other WBC counts indicated loss of immune and other functions in these positive cases comparable to negative patients in our study [6,27,28]. In patients with dengue, atypical lymphocytes were noticed and boost the loss of their immune status. Neutrophils were fragmented in some of the patients' blood smear as reported

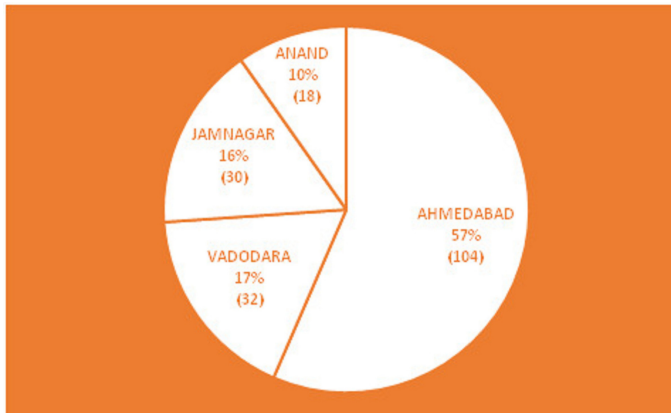


Figure 1: Distribution of dengue positive cases (184) within the four districts of Gujarat (Oct 2016–Dec 2019).

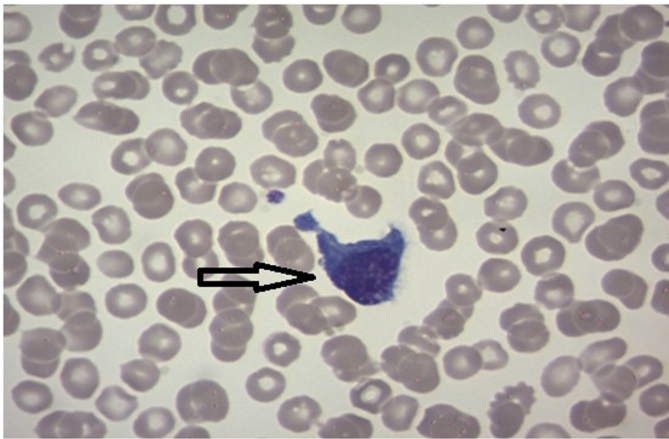


Figure 2: Atypical lymphocytes (→) observed in blood smear of dengue positive patients.

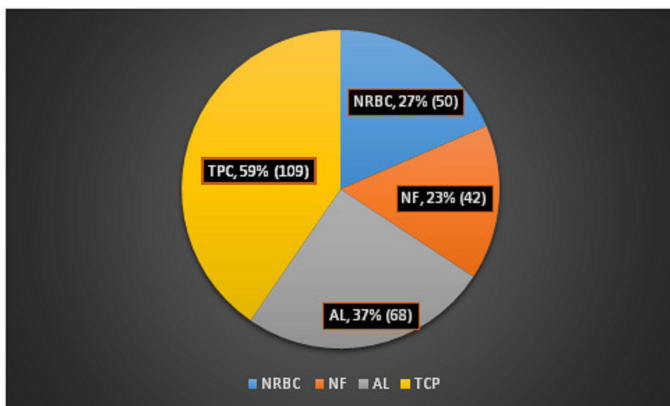


Figure 3: Abnormal blood cell type in positive cases (184). NRBC = Nucleated RBC; NF = Neutrophil Fragmentation; AL = Atypical Lymphocytes; TPC = Thrombocytopenia.

by our pathologists. Few blood smear films exhibited nucleated RBC (NRBC) and hypochromic cells. All these abnormalities of blood cells presented bone marrow cell differentiation arrest or irregularity, display of hypocellularity probably by dengue

virus, and its antigen interplay with progenitor cells [12]. Some researchers [12,27] extensively worked on morphological features of blood cells in dengue-infected patients as well as other viral infection, and they proposed that the presence of thrombocytopenia and atypical lymphocytes are an important diagnostic clues for exact early and alternative diagnosis for dengue infection to support of our study, although molecular detection method (RT-PCR) is to be a gold standard technique for it at present. Thus, our study cohort revealed that dengue disease prevailed in Gujarat and continued to be high till 2019 and depended on region, season, age, gender and other environmental conditions as suggested by others [29–31].

5. CONCLUSION

The data of our study clearly demonstrated that dengue infection is caused by mosquito bites affecting the humans. A careful study of dengue virus is mapped to understand the spread of DF, DHF, and DSS. In Gujarat, its prevalence, incidence, and epidemiology is quite high from 2016 to 2019 (39 months) due to several factors. With a total of 184 cases, infections were high in the younger age group, males, and Ahmedabad district. This viral disease is supported by increased transaminases, alterations in WBC, and platelets. Morphological features of blood cells highlighted the early dengue symptoms as evidenced by thrombocytopenia, platelet clumping, and atypical lymphocytes in addition to PCR, which is standard. Hence, Gujarat in Western India should follow the proper recommendations of the World Health Organizations [26], otherwise it will play havoc, if immediate control measures are not taken.

6. RECOMMENDATION

This cohort suggests an urgent need to check DF and much care needs to be made available by the health departments to eradicate breeding sites of mosquitoes, vaccination facilities, if any, as well as provide clear environment to the public in future, following stratification of healthcare policies strictly around the globe including India. There is also an urgent need for a long-term vector control strategy to prevent this outbreak.

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8. CONFLICT OF INTEREST

The authors express no conflicts of interests regarding this manuscript.

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