Original Article

A 4-Year Retrospective Study Of Dengue And Chikungunya In Tertiary-Care Hospital Of East Delhi

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Abstract

Backgound

Dengue is a vector-borne disease caused by the dengue virus (DENV, 1–4 serotypes), an arbovirus from flaviviridae family and chikungunya virus is an alphavirus.

Material and Methods

A 4 year retrospective study was conducted from January 2017 to December 2020 to determine the prevalence of the disease among in-patients and OPD patients. NS1 ELISA for invitro diagnostic kit was used to detect anti-dengue immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies from 2017-2018. Bio-Rad Dengue NS1 Ag ELISA kit was employed for 2019-2020.

Results

There were 5110 samples were received of which 1269 (24.8%) were laboratory-confirmed as DEN infections. The most common affected age group was 21-40 years old. In terms of gender distribution females were more affected with both dengue and chikungunya as compared to males. Results showed that 28.74% of patients were seropositive in 2017. The maximum seropositivity was observed in 2018 with 36.97% and minimum seropositivity observed in 2020 with 3.43%.

Conclusion

Further epidemiological studies are required to determine the true burden of these infections.

Keywords:

Dengue, Chikungunya, NS1 antigen, IgM antibodies, ELISA

Introduction

Dengue is a vector-borne disease caused by the dengue virus (DENV, 1–4 serotypes), an arbovirus from flaviviridae family. Dengue has emerged as an important global public health problem, more so in tropical and subtropical regions[1]. It is endemic in over 128 countries, with a disease tally of an estimated 58 million infections annually[2]. The WHO regions of Southeast Asia and the western Pacific represent about 75% of the current global burden of dengue.

Aedes aegypti and Aedes albopictus are the main vectors for dengue virus transmission in India[3]. Since the mid-1990s, India has been experiencing frequent dengue epidemics, especially in urban areas, and progressively spreading to additional cities and rural areas, where it was previously non-existent[4,5]. Unplanned urbanization, climate and environmental transition, inadequate vector control and possible population immunity status have been reported as potential risk factors for the spread of dengue in India[6]. The dengue surveillance systems in India, the National Vector Borne Disease Control Program and state governments, are primarily passive. The reported dengue cases have increased dramatically over last decade from 10,137 during 2006–2008 to 188,401 in 2017[7].

Caused by the four serotypes of the dengue flavivirus and transmitted by mosquitoes, dengue affects an estimated 50-100 million people annually around the world, principally in tropical and subtropical regions[8]. Dengue virus (DEN) causes a spectrum of clinical disease ranging from the self-limited dengue fever, usually accompanied by arthralgia, myalgia, and headache, to dengue hemorrhagic fever (DHF) marked by thrombocytopenia, hemorrhagic manifestations, and increased vascular permeability (plasma leakage), to dengue shock syndrome (DSS), which when untreated may lead to death. The infecting serotype and an individual's previous exposure to other DEN serotypes are known to influence disease severity[9].

Chikungunya virus

Transmission and clinical features

The virus is transmitted by Aedes aegypti. The disease is characterized by fever, crippling joint pains (due to arthritis), lymphadenopathy, conjunctivitis and rash. Arthritis is polyarticular, migratory and oedematious (joint swelling) mainly affecting the small joints of wrists and ankles. Haemorrhagic manifestations are seen in some patients. The fever is typically biphasic with a period of remission after 1-6 days. Most patients recover within a week, except for the joint pain which may last for months. Clinically, chikungunya cannot be differentiated from uncomplicated dengue fever. Only laboratory tests can help in confirmation. Chikungunya is the native word for the disease in which the patient lies 'double up' due to severe joint pains.

Materials and Methods

In the present retrospective study, the number of monthly dengue cases reported at Guru Teg Bahadur Hospital, an 1,800-bed tertiary-care hospital in East Delhi, for a period of 4 years (from January 2017 to December 2020) was obtained. Guru Teg Bahadur Hospital is the largest hospital of the government of the National Capital Territory of Delhi in the Trans-Yamuna Area (East Delhi), with a capacity of 1,800 beds. Of the population of Delhi of 12 million people, 12.07% live in East Delhi. Guru Teg Bahadur Hospital is the only Delhi Government tertiary care hospital in the Trans-Yamuna (East Delhi) area, catering to the population of East Delhi as well as patients from the adjacent districts of Noida, Meerut, Loni, Baghpat, and Bulandshahar. Hence, this hospital handles the majority of the dengue cases in East Delhi, as is reflected in the data from 2015, a total of 1632 samples received for dengue and chikungunya in East Delhi.

This hospital accepts all cases of suspected DF irrespective of severity. In this study, dengue cases were defined and classified according to the National Guidelines for Clinical Management for DF released by the Government of India in December 2014. The guidelines classify dengue into undifferentiated DF and severe DF based on clinical manifestations. Non-severe dengue cases include DF and DHF grades I and II, while severe dengue includes DHF grades III and IV and DSS. The clinical criteria for DF, DHF, and DSS given in the guidelines are as follows:

Clinical features of dengue fever

An acute febrile illness of two to seven days' duration with two or more of the following manifestations: headache, retro-orbital pain, myalgia, arthralgia, rash, and haemorrhagic manifestations.

Dengue haemorrhagic fever

A case with the clinical criteria of DF plus haemorrhagic tendencies (evidenced by: positive tourniquet test or petechiae, ecchymoses, or purpura, or bleeding from mucosa, gastrointestinal tract, injection sites, or other sites) plus thrombocytopenia (<100,000 cells/mm^3) plus evidence of plasma leakage due to increased vascular permeability (manifested by a rise in average haematocrit for age and sex of >20% or a drop of more than 20% in haematocrit following volume replacement treatment compared to baseline or signs of plasma leakage (pleural effusion, ascites, hypoproteinaemia [total serum protein level <6 g/dL]) ≤ 20%.

Dengue shock syndrome

All the above criteria for DHF with evidence of circulatory failure manifested by rapid and weak pulse and narrow pulse pressure (mmHg) or hypotension for age, cold and clammy skin, and restlessness.

The protocol of this study was approved by the University College of Medical Sciences Institutional Review Board. Informed consent was obtained from the study subjects. A diagnosis of dengue was made on the basis of clinical findings and serology. Serological confirmation of DF during the study period was carried out using the following tests:

a) InBios DENV Detect™ NS1 ELISA for invitro diagnostic kit was used to detect anti-dengue immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies from 2017-2018.
b) Bio-Rad Dengue NS1 Ag ELISA kit was employed for 2019.
c) Standard E Dengue NS1 Ag ELISA kit was employed for 2020.

Principle of test

The DENV Detect™ NS1 ELISA is a highly sensitive, rapid and reliable assay. It uses on enzymatically amplified "two-step" sandwich-type immunoassay to detect low levels of NS1 in serum.

In this assay, controls and unknown serum samples are diluted in sample dilution buffer, containing secondary antibody, and incubated in microtitration wells. These wells have been coated with a highly effective NS1 antibody and then blocked. NS1 antigens present in the samples are then, "sandwiched" between the capture and secondary antibodies. The presence of NS1 antigen is confirmed by the colorimetric response obtained using an enzyme-conjugate-HRP and liquid TMB substrate. Once the reaction is stopped, using an acidic solution, the enzymatic turnover of the substrate is determined by absorbance measurement at 450 nanometer. The values obtained for the negative and positive sera serve as guidelines as a determining if a sample contains NS1 antigen.

Epidemiology of Chikungunya

The virus first appeared in India in 1963 when it caused extensive epidemics in Calcutta, Madras and other areas. Chikungunya outbreaks have occurred along the east coast of India and in Maharashtra till 1973. It appeared in 2006, when a large outbreak occurred involving Andhra Pradesh, Tamil Nadu, Kerala, Karnataka and Delhi. The disease appears in epidemics after a gap of decade or two. There is no animal reservoir for the virus.

At present, chikungunya is endemic in many states of India such as Karnataka, Andhra Pradesh, Tamil Nadu and West Bengal.

Ethical approval

This study included retrospective analysis of inpatients and OPD patients presented at GTB Hospital for dengue and chikungunya disease evaluation and treatment. The documented data presented in SLR, virology laboratory were used. No personal level data were collected or assessed for the study.

Statistical analyses

The distribution of dengue cases during January 2017 to December 2020 was assessed by $\chi 2$ test. The p-value < 0.05 and CI 95% were considered in all analyses.

Results

Over a period of 4 years, a total number of 5110 samples of inpatients and outpatients for dengue fever, including severe forms of disease such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) and chikungunya received in Guru Teg Bahadur Hospital. The number of reported dengue cases varied by year. Over the study period, the highest number of dengue cases was reported in 2018 (n=1852). Every year, the occurrence of dengue cases displayed a particular pattern. During the pre-monsoon season, hardly any cases of dengue were reported. Most of the cases were reported during the post-monsoon period each year, except in 2018, when the highest number of cases was reported during the monsoon. The average number of dengue and chikungunya cases per month (January to December) over the 4-year period (2017 to 2020) to assess their influence on the occurrence of DF as shown in Table 1 to Table 5.

Table 1 Total samples and positive cases reported in each year

Year	Total samples	IgM Dengue (Positive samples)	Chikungunya IgM (Positive samples)	NS_1Ag ELISA (Positive samples)
2017	1454	385	2	31
2018	1852	393	1	100
2019	1542	440	3	5
2020	262	6	2	1

Table 2 Total samples of Dengue and Chikungunya in 2017

Months	IgM Dengue (2017)			Chikungunya IgM (2017)			NS_1Ag ELISA (2017)		
	Sample received and tested	Positive	Cumulative Grand Total	Sample received and tested	Positive	Cumulative Grand Total	Sample received and tested	Positive	Cumulative Grand Total
Jan	11	Nil	11	1	Nil	1	2	Nil	2
Feb	4	Nil	15	1	1	2	2	Nil	4
March	16	2	31	Nil	Nil	2	3	Nil	7
April	2	Nil	33	Nil	Nil	2	Nil	Nil	7
May	14	2	47	Nil	Nil	2	Nil	Nil	7
June	30	Nil	77	1	Nil	3	5	Nil	12
July	69	10	146	2	Nil	5	28	Nil	40
Aug	163	37	309	3	Nil	8	64	5	104
Sept	211	81	520	1	Nil	9	45	13	149
Oct	410	129	930	1	Nil	10	17	3	166
Nov	252	117	1182	1	1	11	43	10	209
Dec	54	7	1236	Nil	Nil	11	9	Nil	218
	Total patients: 1236	**Positive: 385**		**Total patients: 11**	**Positive: 2**		**Total patients: 218**	**Positive: 31**	

Total patients in 2017: 1454; Male: 652; Female: 802. Percentage of positive samples: 28.74%

Table 3 Total samples of Dengue and Chikungunya in 2018

Months	IgM Dengue (2018)			Chikungunya IgM (2018)			NS_1Ag ELISA (2018)		
	Sample received and tested	Positive	Cumulative Grand Total	Sample received and tested	Positive	Cumulative Grand Total	Sample received and tested	Positive	Cumulative Grand Total
Jan	Nil	Nil	Nil	Nil	Nil	Nil	42	Nil	42
Feb	Nil	Nil	Nil	Nil	Nil	Nil	34	Nil	76
March	Nil	Nil	Nil	Nil	Nil	Nil	33	Nil	109
April	11	Nil	11	Nil	Nil	Nil	Nil	Nil	109
May	42	Nil	53	1	Nil	1	Nil	Nil	109
June	19	Nil	72	Nil	Nil	1	9	1	118
July	48	Nil	120	1	Nil	2	21	Nil	139
Aug	78	3	198	1	Nil	3	32	3	171
Sept	216	43	414	3	Nil	6	99	10	270
Oct	518	205	932	2	1	8	180	62	450
Nov	264	112	1196	Nil	Nil	8	66	24	516
Dec	140	30	1336	Nil	Nil	8	Nil	Nil	516
	Total patients: 1336	**Positive: 393**		**Total patients: 7**	**Positive: 1**		**Total patients: 516**	**Positive: 100**	

Total patients in 2018: 1852; Male: 740; Female: 1112. **Percentage of positive samples: 36.97%**

Table 4 Total samples of Dengue and Chikungunya in 2019

Months	IgM Dengue (2019)			Chikungunya IgM (2019)			NS_1Ag ELISA (2019)		
	Sample received and tested	Positive	Cumulative Grand Total	Sample received and tested	Positive	Cumulative Grand Total	Sample received and tested	Positive	Cumulative Grand Total
Jan	68	9	68	Nil	Nil	Nil	Nil	Nil	Nil
Feb	39	1	107	Nil	Nil	Nil	Nil	Nil	Nil
March	Nil	Nil	107	Nil	Nil	Nil	17	Nil	17
April	Nil	Nil	107	Nil	Nil	Nil	36	Nil	53
May	Nil	Nil	107	Nil	Nil	Nil	41	Nil	94
June	50	1	157	Nil	Nil	Nil	6	Nil	100
July	117	24	274	Nil	Nil	Nil	Nil	Nil	100
Aug	160	22	434	Nil	Nil	Nil	Nil	Nil	100
Sept	322	106	756	Nil	Nil	Nil	Nil	Nil	100
Oct	322	139	1078	1	Nil	1	Nil	Nil	100
Nov	291	134	1369	1	1	2	Nil	Nil	100
Dec	14	4	1383	2	2	4	59	5	159
	Total patients: 1383	**Positive: 440**		**Total patients: 4**	**Positive: 3**		**Total patients: 159**	**Positive: 5**	

Total patients in 2019: 1542; Male: 692; Female: 850. Percentage of positive samples: 32.39%

Table 5 Total samples of Dengue and Chikungunya in 2020

Months	IgM Dengue (2020)			Chikungunya IgM (2020)			NS_1Ag ELISA (2020)		
	Sample received and tested	Positive	Cumulative Grand Total	Sample received and tested	Positive	Cumulative Grand Total	Sample received and tested	Positive	Cumulative Grand Total
Jan	Nil	Nil	Nil	33	Nil	33	1	1	1
Feb	Nil	Nil	Nil	38	Nil	71	1	Nil	2
March	Nil	Nil	Nil	64	Nil	135	Nil	Nil	2
April	Nil	Nil	Nil	22	Nil	157	Nil	Nil	2
May	Nil	Nil	Nil	18	Nil	175	Nil	Nil	2
June	Nil	Nil	Nil	4	Nil	179	Nil	Nil	2
July	Nil	Nil	Nil	7	Nil	186	Nil	Nil	2
Aug	Nil	Nil	Nil	18	2	204	Nil	Nil	2
Sept	2	Nil	2	22	Nil	227	1	Nil	3
Oct	35	6	37	Nil	Nil	227	Nil	Nil	3
Nov	9	Nil	46	18	Nil	295	1	Nil	4
Dec	Nil	Nil	46	14	Nil	259	Nil	Nil	4
	Total patients: 46	**Positive: 6**		**Total patients: 128**	**Positive: 2**		**Total patients: 4**	**Positive: 1**	

Total patients in 2020: 262; Male: 118; Female: 144. Percentage of positive samples: 3.43%

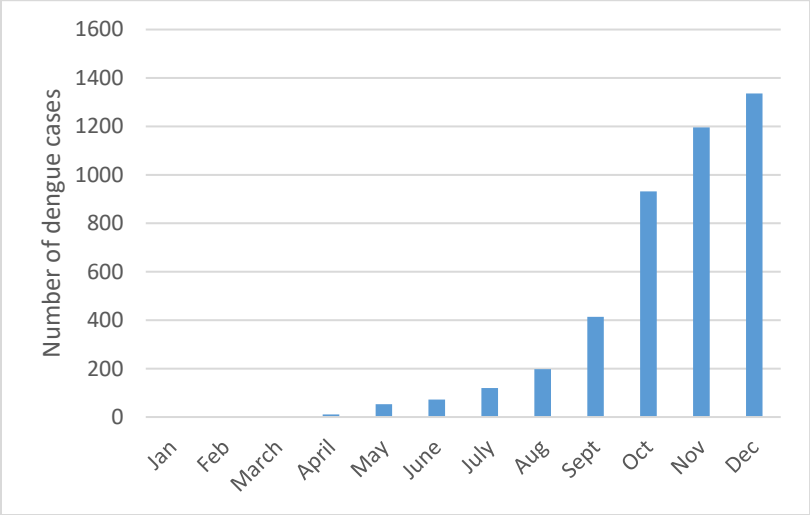

Figure 1 Number of dengue cases presented in 2018

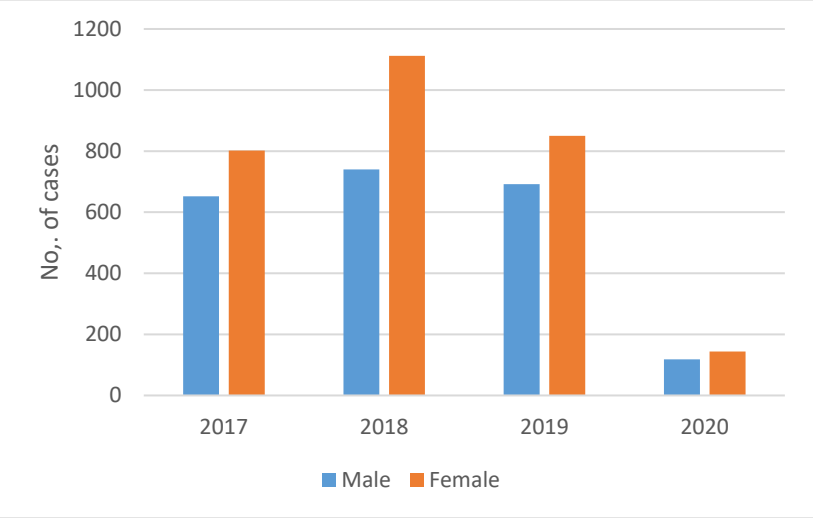

Figure 2 Graphical representation of male to female pattern each year

Discussion

In this study over the 4 years from Jan 2017 to December 2020, a total of 5110 samples were received for dengue IgM antibodies and chikungunya. Overall, 5110 suspected dengue cases were enrolled, and 1269 (24.8%) were laboratory-confirmed as DEN infections. These two viruses may coexist and can be passed from one person to another simultaneously[10]. Both the dengue and chikungunya viruses have been found to contain mutations across their genomes as well as changes in their genotypes. The patient has to have an accurate diagnosis of infection made as soon as possible for the appropriate treatment to be administered[10,11]. Results showed that 28.74% of patients were seropositive in 2017. The maximum seropositivity was observed in 2018 with 36.97% and minimum seropositivity observed in 2020 with 3.43%.

Overall, the seroprevalence of prior dengue virus and chikungunya infection was moderate in the previous studies. As observed

in the different studies from different geographical locations, the seroprevalence of dengue and chikungunya varied by location and year of study.

According to the Sathish et al.[12] study, dengue prevalence was detected at 11% by NS1 antigen detection, which is similar to the study done by Nissi Mathew et al.[13], which indicated an 11.6% prevalence. In the present retrospective study, the prevalence of dengue infection was determined by the detection of IgM antibodies at 7.3 percent, but a study conducted by Nepal H.P et al. found an 8.5 percent prevalence[14]. A study conducted by Sathish et al.[12] showed that the prevalence of chikungunya was 12.7 percent, and another study conducted by Ms. Akanksha Tomar et al. showed that it was 16 percent[15]. The detection of the NS1 antigen is important for the early and rapid detection of infection because it takes place before antibodies develop[10,15].

In the study by Birder et al., there were 284 samples, and 58 of them (20.42%) were positive for one or more dengue serological markers. The youngest patients, those aged 0 to 15, made up the lion's share of the total (48.27 percent)[16]. In the present study, the prevalence of dengue was 114 (3.8 percent), and there were 2867 positive and negative samples (96.17 percent). Those between the ages of 0 and 20 had a prevalence of 25.4 percent, while those between the ages of 41 and 60 had a prevalence of 37.7 percent. Those aged over 65 had a prevalence of 5 percent (44.38 percent).

Among the various social factors, age is one of the characteristics most related to DENV infections[17-19]. In East Delhi, during the 4 years studied, we observed that the majority of dengue cases was reported in individuals aged between 20 and 40 years old. In our study, females were the most affected by DENV infections throughout the study period. This finding have not been observed in other studies.

In Lahore, Pakistan, a cross-sectional descriptive study found that the majority of individuals infected with DENV were males[20].

One of the limitations of the study was that we did not have access to information about the economic status and educational level of individuals diagnosed with dengue and chikungunya.

Over the 4 years, we observed that the majority of dengue cases was without clinical signs. The epidemiological profile described above was clearly observed in 2015. In the following years, 2017 and 2020, there was a more uniform distribution of the less severe dengue phenotypes in relation to at least one of the characteristics evaluated.

Conclusion

India is a high-risk area for arboviruses such as dengue and chikungunya, which share a mosquito vector especially in Trans-Yamuna area of East Delhi. The presence of both the vector and a large population at risk are major contributors to the country's frequent outbreaks of these viral illnesses. Heavy rains have caused stagnant water, which is a favourite breeding ground for the 'Aedes' mosquito, which causes dengue and chikungunya. Both exhibit fever, fatigue, muscle pains, and body aches. The post-monsoon and early winter seasons favour mosquito breeding, and the simultaneous circulation of both viruses has led to an increase in the incidences of dual infection. Dengue and chikungunya infections exist in our areas, and the detection of NS 1 antigen and IgM antibodies helps to determine the prevalence of the viruses in our communities and aids in the early detection of appropriate treatment. Control measures, as well as public awareness, can be implemented.

Finally, although there are specific individual and social characteristics capable of influencing dengue infection and/or its severity, it is important to highlight that there is no universal epidemiological profile of the target population. Therefore, it is necessary that each region, especially the endemic areas, knows in detail the characteristics of its population and periodically update this

information, so that public health policies can be directed to each social reality.

Declaration of conflicts of interest: The authors have no conflict of interest to declare for this study.

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