Original Research Article

Changing Circulating Dengue Virus Serotypes In Malwa Region of Punjab, India During 2019–2021- An Observational Study

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Abstract:

Dengue is the fast evolving mosquito-borne viral diseases which pose a significant public health problem in developing countries. India is among one of the dengue endemic country having co-circulation of all four-dengue virus (DENV) serotypes. This retrospective study has focused on the epidemiological and serotypic distribution trends of DF outbreaks that occurred in Punjab during systematic surveillance from 2019 to 2021. A total of 400 acute serum specimens collected during 2019-2021 were analyzed for serotype analysis by TaqMan Real Time RT-PCR. The predominance of DENV-2 (80%) was observed in 2019 that follow the dwindling pattern. A decreasing trend of DENV-2 population was seen in samples under study and it is replaced by DENV-3 from 2019-2021. Incidence of DENV-4 is sporadic among the study population. Coinfection with more than one serotype was witnessed in the present study. DEN-1,3 was seen to be the most common dual infection constituting 81% incidence rate (39/48 cases) during 2020 and 2021. The change in serotype in a particular geographical region might attribute to the risk of increased frequency and magnitude of outbreaks in the future.

Keywords:

Dengue Fever; Serotype; Outbreak; Real Time RT PCR; NS1Ag ELISA

Introduction

Dengue is the fastevolving mosquito-borne viral disease. The spread of viral disease to homosapiens sapiens occurs through mosquitoes like Aedes aegypti and Aedes albopictus, thus, these infections are also referred as arboviral infections. Dengue virus (DENV) is a single stranded RNA virus belongs to Flaviviridae family. It is divided into four distinct (DENV-1, DENV-2, DENV-3, and DENV-4) serotypes based on their antigenicity resulting into diseased condition (Saha et al., 2016).DENV may infects many organ systems including dermatologic, neurologic, respiratory, gastrointestinal, cardiac, lympho-reticular, renal, and ocular systems leading to undifferentiated fever, dengue fever (DF), dengue shock syndrome (DSS) and dengue hemorrhagic fever (DHF) (Bakshi et al., 2018). It established as DF and advanced into DHF/DSS, which are lethal if untreated. DHF and DSS are typically observed during secondary heterotypic infection and in infants born to dengue-immune mothers (Sankar et al., 2012). All four serotypes can cause the full spectrum of clinical manifestations to range from asymptomatic, mild flu-like symptoms to severe life-threatening dengue complications and have become a global burden ascases are increasing atalarming rate (Wahyono et al., 2017).WHO reported that the number of dengue cases increased over 8 fold in the last two decades; from 505,430 cases in 2000 to over 2.4 million in 2010 and 5.2 million in 2019.There was an increase in total deaths from 960 to 4032 from2000 to 2015 (Karyanti et al., 2014).

The Indian encounter with this disease is captivating as cases expanded intensely over the last few decades, with 34% of total global cases at regular intervals from almost all parts of India. The first confirmed outbreak of dengue fever in India occurred in Kolkata in 1963–1964 (Chaturvedi UC et al. 2008, Sarkar A et al. 2012). It spread towards northern parts of the country and reached Delhi in 1967 (Gupta

Net al. 2012). In due course the first major nationwide outbreak spreads throughout the entire country, in the year 1996 (Chaturvedi UC et al. 2008, Dar L et al. 1999, Kaur H et al. 1997). Delhi has become hyperendemic for dengue from northern India with outbreaks occurring frequently, the last reported in 2006. Punjab being neighbouring state has also come as hyperendemic region for this disease in 2015, 2017 and 2018 (Jiménez - Silvaet al., 2018). Major or minor outbreaks of DF/DHF were reported due to favourable conditions like rapid urbanization, favorable temperature and rainfall conditions that lead to mosquito proliferation and transmission of disease (Megawati et al., 2017).Hence, it becomes crucial to develop comprehensive epidemiological control measurements of DENV from suspected human specimens primarily in the transmission season. Therefore, the present study was planned to compare the pervasiveness of DENV serotypes within dengue suspected febrile illness cases reported to a tertiary care hospital for a dengue fever reported from Southeast Punjab during 2017-2020. The study also concentrated to recognize the altering pattern of dengue serotypes especially during outbreak for early public health awareness and to overcome the adversities appearing during outbreak as multiple dengue virus serotypes circulate in this region.

Materials and Methods:

This retrospective study was performed from the whole blood samples among clinically suspected dengue patients (n=400) reported to departments of Medicine & Pediatrics from a tertiary care hospital in Punjab during the time span of 2019-2021. All the serum samples were tested for the presence of anti-dengue IgM using MAC ELISA (National Institute of Virology, Pune) and/or NS1Ag ELISA kit (QIAamp viral RNA mini kit (QIAGEN, Germany) according to the manufacturer's instructions. The positive control RNA mixtures of DENV-1 to DENV-4 were procured from NIV Pune. Nuclease free water was used as negative control. RNA was eluted in 50 µl Elution buffer.The extracted RNA from clinical samples was subjected to DENV fourplex Taqman Real-Time RT-PCRas reported earlier by Johnson et al., 2005 with little modification.The sequence of primers and probes used in the present study are shown in (Table 1)

Table 1 : The sequence of Primers and Probes used in the present study

Primer	Reporter Dye	Sequence (5' -3')	Quencher
D1-F		CAAAAGGAAGTCGTGCAATA	
D1-R		CTGAGTGAATTCTCTCTACTGAACC	
D1-Probe	FAM	CATGTGGTTGGGAGCACGC	BHQ1
D2-F		CAGGTTATGGCACTGTCACGAT	
D2-R		CCATCTGCAGCAACACCATCTC	
D2-Probe	TAMRA	CTCTCCGAGAACAGGCCTCGACTTCAA	BHQ1
D3-F		GGACTGGACACACGCACTCA	
D3-R		CATGTCTCTACCTTCTCGACTTGTCT	
D3-Probe	TEXAS RED	ACCTGGATGTCGGCTGAAGGAGCTTG	BHQ2
D4-F		TTGTCCTAATGATGCTGGTCG	
D4-R		TCCACCTGAGACTCCTTCCA	
D4-Probe	CY5	TTCCTACTCCTACGCATCGCATTCCG	BHQ3
RP-F		AGATTTGGACCTGCGAGCG	
RP-R		GAGCGGCTGTCTCCACAAGT	
RP-Probe	FAM	TTCTGACCTGAAGGCTCTGCGCG	BHQ1

(Haryanto et al., 2015).The reaction mixture includes 200nM (each) of DEN-1 and DEN-3-specific primers, 100 nM (each) of DEN-2 and DEN-4-specific primers, 180 nM each probe and 0.5 µl of Super-Script III RT/Taq mix and 5 µl RNA (isolated from NS1 ELISA positive samples) in a 25-µl volume of the total reaction mixture. Taqman Real-Time RT-PCR amplification was performed on the ABI 7500 real-time PCR system (std.) platform at 50°C for 30 min, 95°C for 10 min and 45 cycles of amplification with 95°C for 15 sec and 60°C for 1 min. Amplification curves rising above the background noise and intersecting the threshold below 37 cycles were termed as positive for the presence of any dengue serotype.

Statistical Analysis:

GraphPad Prism version 5 was used to analyze data collected during the present study. Categorical variables like gender, the spatial distribution of dengue cases, serotype distribution were expressed as percentages. The significance of categorical variables was analyzed by Pearson's $\chi2$ testor Fisher's exact test. Continuous variables like age were investigated by central tendency measures and expressed as median [25-75%] Interquartile range(IQR). The normality check for continuous variables was done by the Kolmogorov-Smirnov test. The continuous variable data was analyzed by One-Way ANOVA. The analyses were performed at 95% confidence. The P values less than 0.05 was taken as statistically significant.

Results:

Demographic features of the Dengue cases: The present work was carried out on patients whose sample collections were made at tertiary care hospital during 2019 to 2021. The 400 samples were screened with IgM antibody/NS1Ag ELISA which resulted in 273 being positive.The mean age of the studied population is 35 years with median (IQR) of 30years in dengue positive patient. Most of the patients detected with dengue consisted of young group ageing between 21-30 years, followed by the 31-40 years age group. The data were analyzed for year wise distribution by one-way ANOVA and considered highly significant (p<0.01) for the 21-30 years age group. Here, we also intended to compare the gender wise distribution of dengue fever and found that males were more infected by the disease (Table 2).

Table 1 : The sequence of Primers and Probes used in the present study

Age Group	Male	Female	p value	Urban	Rural	p value
0-10 years	2 (1.2%)	0	<0.01	2 (1.2%)	0	<0.0001
11-20 years	18 (10.6%)	10 (9.7%)		8 (4.6%)	20 (20%)	
21-30 years	67 (39.4%)	43 (41.7%)		72 (41.6%)	38 (38%)	
30-40 years	51 (30%)	36 (35%)		66 (38.2%)	21 (21%)	
40-50 years	25 (14.7%)	8 (7.8%)		17 (9.8%)	16 (16%)	
>50 years	7 (4.1%)	6 (5.8%)		8 (4.6%)	5 (5%)	
Total	170	103		173	100	

Also Urban population seems to be more prone to this disease as compared to rural and this difference is highly significant (p<0.0001).

Distribution of DENV Serotypes during 2019-2021:

NS1Ag ELISA test results shown in (Table 3)

Table 3: Prevalence of DENV serotypes in Punjab, India during 2019-2021.

Year	No. of dengue positive samples	DENV-1	DENV-2	DENV-3	DENV-4
2021	118	32 (27%)	44 (37%)	38 (33%)	4 (3%)
2020	120	47 (39%)	45 (38%)	16 (13%)	12 (10%)
2019	35	0	28 (80%)	7 (20%)	0
Total	273	79 (29%)	117 (43%)	61 (22%)	16 (6%)

revealed that out of 273 cases, the maximum no. of cases were infected with DENV-2, followed by DENV-1 and DENV-3 whereas least number of dengue patients was infected with DENV-4 serotype with only 16 cases. In addition to this, co-infection with two dengue serotypes was observed in few cases. Co-infection of DENV 1 and DENV 3 was found to be more prevalent in patients in comparison to DENV 1 & 2 and DENV-2 & 3 as shown in (Table 4).

Table 4: Incidence of co-infection with dengue viruses in Punjab, India during 2019-2021

Year	Total No. of Co-infection cases	DENV-1+ DENV2	DENV-1+ DENV3	DENV-2+ DENV-3
2021	20	6 (30%)	11 (55%)	3 (15%)
2020	28	0	28 (100%)	0
2019	0	0	0	0
Total	48	6	39	3

We also analyzed the year-wise distribution of dengue disease which revealed that DENV-2 was the prevalent serotype followed by DEN-3 during 2019. However, no case of DENV 4 serotype was detected in 2019. All the four serotypes were in co-circulation during 2020 in this region with the predominance of DENV-1 (39%) followed by DENV-2 (38%), DENV-3 (13%), and DENV-4 (10%). In the years 2019 and 2020, the predominance of DENV-2 was observed. The DENV-2 completely masked all other serotypes prevalent in this region during the study period.The year-wise distribution of DENV-serotypes from 2019 to 2021 presented here is statistically highly significant ($p<0.0001$). The presence of concurrent infections by DENV serotypes is quite noticeable in the population under study. The overall incidence of dual infections was 18% (48/273). DEN-1,3 was seen to be the most common dual infection constituting 81% incidence rate (39/48 cases) during 2020 and 2021followed by DEN1,2 (13%) and DEN 2,3 (6%) each from 2019 to 2021.

Discussion:

Dengue (DEN) is the widely distributed viral disease with an estimation of 50 million DEN infections occur annually and approximately 2.5 billion people live in DEN endemic Countries (Damodar et al., 2017). In the past few years substantial upsurge in the geographic spread, amount of cases and severity of the disease has been reported due to changes in the environment, rapid urbanization and increase in transportation facilities among various regions of India (Chakravarti et al., 2012). Ours is a tertiary care hospital with thickly populated catchment areas andhugenumbers of samples are obtained in this hospital. Hence, the serotypic records from these samples may reveal the flowing serotype in Punjab and adjacent areas which may benefit to develop an effective regulator and management policies against the dengue threat.

Among the four serotypes of DEN our study reported the high prevalence of DENV-3 (42%) and DENV-1 (32%) during 2017, while all the four serotypes were in co-circulation during 2018. DENV-1 and DENV-3 were the most common serotypes observed during the epidemic phase (2017 and 2018). This study reflects a positive correlation between the co-circulation of different serotypes with dengue epidemic outcomes. Reports have shown that although all the four serotypes had been circulating during an outbreak, one serotype usually dominated a particular outbreak (Wijayanti et al., 2016). In an another study it was reported that DENV1 and DENV3 were present as the leading serotype in an outbreak during 1999 and 2005 in New Delhi (Fahri et al., 2013).In 2019 DENV-2 (80%) was the predominant serotype in the present studied population group. In support to the present study, Rao in 2020 also found that DENV-2 was associated with outbreaks in Delhi, Jammu, Haryana, Lucknow, and Gujarat during the period of 2018 as reported by present study (Rao et al., 2020).

It has been shown in a previous study that DENV-3 and DENV-1 are more often associated with primary infection, whereas DEN-2 and DENV-3 serotypes are the most common cause of DHF during secondary infections (Haryanto et al., 2016).The incidence of monotypic infections was higher than the concurrent infections. DENV-1,3 was the predominant dual infection reported from our study, while DENV-1,2 and DENV-2,3 were sporadically present. It has been suggested from the previous studies that co-infections affect the disease progression since multiple serotypes infection stimulates the risk of emergence of new virulent strains (Suppiah et al., 2018).Earlier the co-circulation of all the four serotypes in a particular outbreak has been reported in Karnataka (42.9%), Hyderabad (45.4%) and other northern or eastern parts of India. These regions with circulation of more than one serotype simultaneously are of high significance as they are more prone to severe dengue infection (Vinodkumar et al., 2013; Vaddadi et al., 2017). Gupta E et al., in 2006 reported the cocirculation of all the four dengue serotypes in the neighbouring State of Delhi in the year 2003. Another studies conducted by Reddy et al., in 2017 and Racherla et al., in 2018 also reported the co-circulation of all four with high percentage of DENV 2 and DENV 4 respectively. Thus this study highlights the importance of different DENV serotypes and there circulation which may bear the potential of causing severe outbreak. Therefore, continuous surveillance is recommended for understanding the epidemiology of the diseases and monitoring the changes in the characteristics of circulating DENV strains.

Limitation of the study:

The limitation of the current study is that it reflects the data collected only from a tertiary care hospital in Punjab. So, there is a need of continuous surveillance to detect circulation pattern of DENV-serotypes from this region which will help the authorities in formulation of sound control measurements early in transmission season and better patient management.

Conclusion:

The study has presented the circulation trend of different DENV serotypes in Punjab, India, from 2017- 2020. DENV-3 was the most prominent serotype followed by DENV-1 during the initial phase of the study (in 2017-2018). The incidence of co-infections is quite noticeable in this region. A sudden increase in DENV-2 cases was observed in 2019. The shift from one serotype to another and co-circulation of multiple DENV serotypes leads to more frequent outbreaks and suggests that Punjab is on the verge of becoming the hyperendemic state for dengue infections. Elaborated monitoring on the circulating serotypes and genotypes of DENV and their effect on disease severity is highly required to help improve patient management and control measures taken by public health authorities.

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Conflicts of Interest: Nil

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