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Serotype Variations and Distributions of Dengue Virus During the 2022 Outbreak in Chattogram, Bangladesh

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Abstarct:

Introduction: In the year 2022, a Dengue virus outbreak occurred nationwide in Bangladesh, including Chattogram, a port city. The purpose of this study was to identify and document the serotypes of Dengue virus and to measure the viral load of the virus positive specimens.

Methods: This observational study was conducted in a tertiary care hospital of Chattogram during a period of six months from June through December, 2022. A total of 74 patients with symptoms suggestive of Dengue Fever(DF) and NS1 or IgM/IgG positive dengue patients were recruited into the study. From them clinicopathological data was collected on a case record form(CRF). Ten ml of venous whole blood was drawn in EDTA tubes then centrifuged and was sent to the laboratory at SlieaGen L.L.C in Austin, Texas, USA, for Dengue serotyping and viral load analysis by qPCR-LDT method. A total of 73 specimens were analyzed, one specimen was excluded. Ethical clearance was obtained from the hospital ethical review board, written informed consents were also taken from all subjects. Serotyping and qPCR testing data was shared by SlieaGen LLC once analysis was completed.

Results: Amongst the 74 patients most were male 57(77%) and most [20(40.5%)] patients were young at 21-30 years. All had history of fever(100%), followed by body aches 67(90.5%), headache 49(66.2%) and weakness 55(74.3%). Positive NS1, IgM Dengue Ab and IgG Dengue Ab were found in 68(91.9%), 8(10.8%) and 3(4.1%) cases respectively. Frequency of serotype distributions revealed DENV 1 and DENV 3 were the most common serotypes 25(33.8%) and 16(21.6%) respectively also DENV 2 was 3(4.1%) and DENV 4 was 1(1.4%). Among 27 virus positive cases single infection DENV 1 was found in 10(13.51%) samples, DENV 3 and DENV 4 found in 1(1.4%) samples each, doubly infected (DENV 1 + DENV 2) was found in 12(16.2%) samples, triple infected (DENV 1 + DENV 2 + DENV 3) was found in 3(4.1%) samples. No virus was detected in 46(62.16%) samples. Viral load (Mean ± SD) found in DENV 1(890974.48 ± 4156206)cp/ml, DENV 2 (33423.00 ± 9088.35)cp/ml, DENV 3(23991.12 ± 51250.18)cp/ml and DENV 4 (822.00cp/ml) in virus detected samples

Conclusions: All four serotypes were found in the 2022 Dengue outbreak in Chittagong and DENV 1 and DENV 3 were common serotype. Simultaneous double and triple serotype infection in the same patients may be related with different unusual clinical features which need further documentations in future.

Key words: Dengue, Serotype, Viral load

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Introduction:

Dengue, the fastest-spreading mosquito-borne infectious disease, has emerged as a global public health problem and WHO considers it a global threat[1]. This is particularly concerning as no specific treatment or vaccine against dengue is not widely available. Furthermore, the dengue virus's genetic heterogeneity (serotype and genotype) adds another dimension to the public health challenge due to the increased risk of disease severity (secondary and tertiary infection). Dengue has spread over 125 countries, with 400 million annual infections and 40,000 deaths[1]. The endemic regions of tropical and subtropical countries (South East Asia and South Asia) account for 70% of the dengue burden[1].

Arbovirus called Dengue virus under Flavivirus genus and transmitted by Aedes mosquito. There are four serotypes of dengue virus Den -1 to Den -4. There are different genotypes of all four of these serotypes. Clinical presentations and severity related with the serotype involvement and multiple serotype infection in the same patient at a point of time causes worse outcome[1,2].

There are paucity of data regarding analysis of serotype distribution among the patients of dengue affecting the patients in Bangladesh. So present study was designed to find the serotype variations and distributions among the dengue patients occurring during the 2022 outbreak of dengue in Chattogram, Bangladesh.

Methods: It was an observational study, done in a tertiary care hospital, Chattogram, Bangladesh in collaboration

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with a research laboratory of Texas, USA. Period of study was six months and duration of sample collection was 5 months from 1/6/22 to 31/11/22 while the outbreak of dengue occurred hugely nationwide including Chattogram. Patients with history suggestive of Dengue with characteristic fever or if researchers clinically suspected the patients have DF in their background history and examination and those were NS1 positive or IgM/IgG positive for Dengue were recruited in the study. During that period a total of 74 NS1 positive or Ig-M/Ig-G positive cases done by ICT (Abbott- Bioline Diagnostic Company Limited – Republic of Korea) were recruited as study sample. 10 ml of venous blood were collected from those positive cases and mixed with EDTA anticoagulant. Then samples were shipped to USA research laboratory for serotype analysis. Dry ice was used to maintain the temperature of the sample below -20º C during transport from Bangladesh to USA. In the testing lab of USA serotypes of Dengue were detected by qPCR-LDT method and identification of viral load in copies/ml were done in 73 samples. One sample was not tested due to technical issues. For the study, ethical clearance was taken from hospital authority, informed written consent was taken from all patients for data and blood collection and FDA approval was taken for the shipment of the samples in USA and funding was done by the research laboratory.

Results:

Table-I

Age and gender distributions of study patients(N-74)

Age group	Gender		Total(N,%)
	Female(N,%)	Male(N,%)	
<20 years	3(17.6%)	11(19.3%)	14(18.9%)
21-30 years	7(41.2%)	23(40.4%)	30(40.5%)
31-40 years	5(29.4%)	17(29.8%)	22(29.7%)
41-50 years	2(11.8%)	4(7.0%)	6(8.1%)
>51 years	0(0%)	2(3.5%)	2(2.7%)
Total	17(22.97%)	57(77.03%)	74(100%)

Table I showing male were affected more then the female (77.03% vs 22.97%) and younger age groups were more vulnerable from 21 years to 40 years

Table-IIa

Clinicopathological data(N-74)

Clinicopathological data		N,%
Fever		74(100%)
Nausea		46(62.2%
Vomiting		41(55.4%)
Bodyache		67(90.5%)
Myalgia		46(62.2%)
Arthralgia		9(12.2%)
Retroorbital pain		9(12.2%)
Headache		49(66.2%)
Skin rash		9(12.2%)
Weakness		55(74.3%)
Abdominal pain		10(13.51%)
Features of shock		8(10.81%)
Positive Tourniquet test		
Platelet count	>50,000/cmm	54(63%)
	<50,000/cmm	20(37%)
Hct	<35%	8(10.6%)
	35-45%	53(71.8%)
	>45%	13(17.8%)
Elevated liver enzymes(ALT and/or AST)		11(14.6%)

Table IIa showing fever, nausea, vomiting, bodyache, myalgia, headache, weakness low platelet and elevated liver enzymes were some common clinical findings among the dengue patients

Table-IIb

Clinicopathological data

Data	Parameter	Results(N,%)
NS_1	Positive	68(91.9%)
	Negative	2(2.7%)
	Not done	4(5.4%)
Ig-M dengue Ab	Positive	8(10.8%)
	Negative	2(2.7%)
	Not done	64(86.5%)
Ig-G dengue Ab	Positive	3(4.1%)
	Negative	7(9.5%)
	Not done	64(86.4%)

Table IIb showing 68(91.9%) were NS1 positive cases and few were IgM or IgG positive cases.

Table-III

Variations of serotypes in the test sample(N-74)

Type of serotypes	Detected	Not detected	Not tested
DENV 1	25(33.8%)	48(64.9%)	1(1.4%)
DENV 2	3(4.1%)	70(94.6%)	1(1.4%)
DENV 3	16(21.6%)	57(77%)	1(1.4%)
DENV 4	1(1.4%)	72(97.2%)	1(1.4%)

Table III showing DENV 1 serotype was found in 25(33.8%) samples, DENV 2, DENV 3 and DENV 4 found in 3(4.1%), 16(21.6%) and 1(1.4%) samples respectively.

Table-IV

Distributions of serotypes in individual test samples(n-74)

Serotype distributions	Number(%)
DENV 1 only	10(13.51%)
DENV 2 only	0(0%)
DENV 3 only	1(1.4%)
DENV 4 only	1(1.4%)
DENV 1+DENV 3	12(16.2%)
DENV 1+ DENV 2 + DENV 3	3(4.1%)
Not detected	46(62.16%)
Not tested	1(1.4%)

Table IV showing distribution of serotypes in individual test samples where DENV 1 only found in 10(13.51%) samples, combined DENV 1 + DENV 3 found in 12(16.2%) samples and combined DENV 1+ DENV 2 + DENV 3 found in 3(4.1%) samples.

Table-V

Viral load in the virus detected samples

Type of DENV	Frequency	Mean ± SD(copies/ml)
DENV 1	25	8909.48 ± 4156206
DENV 2	3	33423.00 ± 9088.35
DENV 3	16	23991± 51250.18
DENV 4	1	822.00

Table V showing copies/ml of virus were detected in all 4 serotypes of dengue virus.

Discussion:

In the present study male were affected more then the female (77.03% vs 22.97%) and younger age groups were more vulnerable which was from 21 years to 40 years. A study done by Uddin et al.[2] in the year 2021 in Bangladesh found that common age group distributions was <20 years 9(27.3%) and 21-30 years 12(36.4%) and mean age was 31.55±16.03 years. Also gender distributions in that study had more male patients affected then female. Similar findings were also found to an old study done by Sharma et al.(1998)[3].

In the present study fever, nausea, vomiting, bodyache, myalgia, headache, weakness low platelet and elevated liver enzymes were some common clinical findings among the dengue patients. Among the 74 cases 68(91.9%) were NS1 positive cases and few were IgM or IgG positive cases. In the study done by Uddin et al.[2] describes common clinical presentations of the study patients where findings were similar with the present findings. They found joint symptoms, fever, headache, GIT upsets and bleeding manifestations. In a study from Nimmannitya et al[4]. around 96% of patients had congested pharynx, and rhinitis was reported in 13% of the patients. Bleeding from various sites was seen much less in the present study. This is in contrast to the finding of Horvath from Australia[5] and Sharma from India[3]who reported 63% and 69% of bleeding episodes respectively.

As of today, only a nationally representative seroprevalence study conducted from 2014 to 2015 revealed that 24% of tested individuals (n = 5866) had a previous history of dengue across the country, and over 80% were seropositive among the study population in two major cities (Dhaka and Chattogram) [6,7]. This seroprevalence study estimated that 40 million individuals had been infected in Bangladesh, with 2.4 million annual dengue cases[6,7].

Now dengue is prevalent for consecutive years. The evidence of dengue spreading in different non-endemic regions is mounting due to three consecutive outbreaks, in 2019, 2021, and 2022. During the 2019 outbreak, 48.4% cases were reported from all 64 districts of Bangladesh. Similar circumstances were seen in the outbreak of 2021, where 20.4% of cases were recorded from locations outside Dhaka [8]. Nevertheless, the true magnitude of the 2021 outbreak (28,429 ca es and 105 deaths) remains unknown as it was masked by the COVID-19 pandemic[9]. In the 2022 outbreak, over one-third (37.5%) of all cases were reported from outside of Dhaka city.

There were different serotypes prevalent in the year 2022 dengue outbreak in Chittagong. Surprisingly all 4 serotypes of DENV were found in the year 2022 dengue outbreak in Chattogram which was unique and alarming for the future. The information on the evolution and diversity of the dengue virus is sparse in Bangladesh let alone in Chattogram city. Most studies described the genotypes of dengue based on small sample size[10]. In early outbreaks (2000–2002), DENV-3 was the prevalent serotype, albeit all four serotypes were circulating. Afterward, there were no data available for serotypes until 2012. Later, Muraduzzaman et al. revealed that DENV-1 and DENV-2 were the prevalent circulating serotypes between 2013 and 2016 [11]. In the present study DENV -1 and DENV-3 were the common serotype in Chattogram outbreak in the year 2022. However, DENV-3 emerged after a hiatus in 2017 with the prevalent serotype of DENV-2. Since the largest outbreak in 2019, DENV-3 has been the most prevalent circulating serotype[12].

Not much information is available on the genotypic variability of DENV. Three genotypes (I, II, II) of DEN-3

and the cosmopolitan genotype of DEN-2 are currently circulating in Bangladesh. Arguably, the emergence of the DENV-4 serotype, which has been missing for more than 20 years, could pose a significant public health threat to Bangladesh because of secondary infection[7,13].

Another notifiable findings in the present study was the combined infections of multiple DENV here DENV-1 + DENV - 3 and DENV-1 + DENV-2 + DENV -3 in the same patients in the same point of time. These may explain the severity of dengue in the patients with significant mortality. Future study is needed to find the consequence and outcome with these type of combined serotypic infections.

Conclusion:

we have showed that a small picture of dengue situations of Chattogram, Bangladesh during the dengue outbreak in the year 2022. We have identified the prevalent of all 4 serotypes in that year and interesting finding was combined infection of multiple serotypes in same patients. Our findings would be helpful for other similar settings elsewhere in the country and in the world.

Conflict of Interest: None

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