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A Study on *Chikv-Denv* Co-Infection in West Bengal, India.

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Abstract
Background: *Chikungunya virus (CHIKV) and dengue viruses (DENV) are both present individually in West Bengal and infection caused by both simultaneously has been recorded since 1965.*
Objectives: *In 2011, a study was carried out to detect co-infection due to both the viruses from 671 blood samples collected from febrile patients attending OPDs and in-patient departments of different tertiary care hospitals of Kolkata and different district hospitals of West Bengal from March 2011-November 2011. The samples were tested for immunoglobulin M (IgM) antibody against both CHIKV and DENV by the enzyme-linked immunosorbent assay (ELISA).*
Result: *493 patients (73.47%) had IgM antibody against CHIKV and 105 (15.65%) patients had IgM antibody against DENV, whereas 41 patients (6.11%) had IgM antibodies against both CHIKV and DENV. Fever, joint pain, rashes, headache, body-ache were the common complaints. Severe arthralgia and joint swelling was complained mainly by the CHIKV-positive cases. Headache, retro-orbital pain and altered sensorium were mainly associated with DENV positive cases. No mortality was observed.*
Conclusion: *Detection of IgM antibodies by ELISA against both appears to play an important role in differentiating between the two and initiating proper treatment.*
Keywords: *Chikungunya, Dengue, ELISA, IgM serology*

I. Introduction

Arthropod-borne viruses are a serious major public health problem worldwide. *Chikungunya virus (CHIKV) and dengue virus (DENV)* are the two most rapidly spreading *arboviruses.* The *CHIKV* belongs to the *Togaviridae* family genus *Alpha virus*, whereas *DENV* belongs to the family *Flaviviridae* and genus *Flavivirus*. Both the viruses are RNA viruses and both spread to man by the vector mosquitoes *Aedes aegypti* and *Aedes albopictus.* [1] Both the diseases have some common signs and symptoms like fever, rash, joint pain, headache, and body-ache.

Dengue is one of the rapidly spreading infections affecting 50 million people per year [2] and mortality rate is 25,000 per year in tropical and subtropical countries. In India, *DENV* was first isolated in Kolkata in 1963. [3]

Concurrent circulation of *CHIKV* and *DENV* is quite common in South-East Asia [4, 5, 6] and since 2005 co-infections have been reported in Delhi, India [7] and in South India. [8]

The proposed community based cross-sectional study was conducted to study the prevalence of dual infection of *dengue* and *Chikungunya viruses* in the same patient from the OPDs and inpatients of the different hospitals of West Bengal, India.

II. Material &Methods

In 2011, a study was conducted from March 2011-November 2011 from patients complaining of high fever (> 39°C) with/without joint pain, rash, joints swelling, headache, and retro-orbital pain attending the OPDs and in doors of different tertiary care hospitals in Kolkata. Outbreaks samples were also collected from the various districts.

Informed consent was taken from the patients/guardians and ethical committee approval was also taken.

About 2 c.c. blood was collected and the sera were separated from the clotted blood samples and stored in aliquots at −20°C. The samples were tested for the presence of any bacteria like typhoid and malaria parasites.

A total of 671 samples were collected. Hb gm%, WBC and platelet counts of the patients was estimated and found to be normal.

All of the samples were subjected to an enzyme-linked immunosorbent assay (ELISA) test to detect the presence of immunoglobulin M (IgM) antibodies against both CHIKV and DENV by IgM antibody-capture (MAC)-ELISA kits. The kits used were from the National Institute of Virology, Pune, India. Optical density (OD) was measured at 492 nm using an ELISA reader.

III. Results

Of the 671 samples, 493 samples (73.47%) had IgM antibodies against *CHIKV* and 105 samples (15.65 %) had IgM antibody against *DENV*. Only 41(6.11%) samples had IgM antibodies against both *CHIKV* and *DENV*. No cross reactivity, however, was observed between the two viruses.

Out of 41 IgM positive dual-infected cases, seven patients [3 male and 4 female] (17.07%) were of the age ≤ 10 years. 15 adults out of 41 patients (36.59%) [5 male and 10 female] of the age-group 31-40 yrs were affected by both the viruses. The female/male ratio was 1.73:1. (TABLE 1) The most probable reason of females being more affected than males is probably because they reside in the house at daytime and hence get more exposed to the vector *Aedes* sp., which is domestic in nature and a day biter. [9, 10]

Table 1: Age and sex-wise distribution of IgM-positive cases in West Bengal, India, 2011

Age-group	Total no. of cases			Total +ve case-***CHIKV***			Total+ve case-***DENV***			Total +ve case-***CHIKV +DENV***		
	Total	M	F	Total	M	F	Total	M	F	Total	M	F
0-10	38	21	17	13	06	07	18	12	6	07	03	04
11-20	71	39	32	37	19	18	26	13	13	05	02	03
21-30	106	43	63	90	37	53	17	02	15	09	02	07
31-40	197	79	118	168	64	104	29	08	21	**15**	05	10
41-50	128	58	70	79	47	32	07	03	04	01	0	01
51-60	83	52	31	65	43	22	07	03	04	02	02	0
61 & >	48	27	21	41	23	18	01	01	0	02	01	01
TOTAL	**671**	319	352	**493**	239	254	**105**	42	63	**41**	15	26

High grade fever ≤ 39 ^{0} C was the commonest presenting feature in both the single and dual virus infection, followed by joint pain, rashes, headache, and severe body-ache. Retro-orbital pain was present in *DENV* positive cases and in 3 dual infected cases. Splenomegaly, diarrhea, altered sensorium and gum bleeding were some of the other complaints. In all cases the OD value of the Chikungunya IgM antibody was at least six times higher than the OD value of the dengue IgM antibody. No mortality was observed and all the patients including the dual infected patients recovered quickly.(TABLE 2)

Table 2:--Clinical features of dual infected patients

FEVER	24	58.54%
BODY ACHE	20	48.78%
RASH	11	26.83%
HEADACHE	12	29.27%
RETRO-ORBITAL PAIN	03	7.32%
POLYARHRITIS	04	9.76%
ALTERED SENSORIUM	02	4.88%
SPLENOMEGALY	02	4.88%
DIARRHOEA	01	2.44%
UTI	01	2.44%
VOMITTING	01	2.44%
WHOLE BODY ACHE	01	2.44%
WEAKNESS	01	2.44%
GUM BLEEDING	01	2.44%

Regarding the monthly distribution of co-infected cases, the highest number of cases was found in the month of August (27/41 i.e. 65.85%) followed by the month of September (07/41 i.e.17.07%). The stagnant fresh water during the rainy seasons (June to September) favored the breeding of the vector mosquitoes and hence the co-infected cases attained its peak in the month of August and September, which is the post-monsoon period. (TABLE 3)

Table 3: Monthly distribution of *Chikv*, *Denv* & dual infection

MONTHS	*CHIKV*		*DENV*		DUAL INFECTION	
	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE
MARCH	0	1	0	1	0	0
APRIL	3	2	2	0	0	0
MAY	1	2	1	0	0	0
JUNE	3	2	2	2	1	0
JULY	7	3	7	12	0	3
AUGUST	15	19	6	12	**9**	**18**
SEPTEMBER	34	36	8	12	4	3
OCTOBER	70	86	8	12	0	1
NOVEMBER	98	111	8	12	1	1
	239	254	42	63	15	26
TOTAL	**493 (73.47%)**		**105 (15.65 %)**		**41 (6.11%)**	

Regarding the districtwise distribution of all the cases—CHIKV positive, DENV positive and dual infection positive, residents from Kolkata were more affected (17/41—41.46%) than other districts by both the single virus and dual virus. This can however be explained by the fact that residents of Kolkata has more access to reporting to hospitals in comparison to the residents of the districts.

Table 4: Districtwise distribution of *Chikv*, *Denv* & dual infection

DISTRICTS	*CHIKV*	*DENV*	DUAL INFECTION
24Pgs (N)	41	2	3
24Pgs (S)	10	3	2
Hooghly	12	24	5
Midnapore(E)	2	2	0
Midnapore(W)	2	2	0
Malda	3	0	0
Murshidabad	3	0	3
Purulia	3	0	0
Nadia	7	3	1
Bankura	4	6	2
Birbhum	11	16	3
Howrah	35	28	4
Burdwan	2	1	1
Kolkata	358	1	17
TOTAL	**493**	**105**	**41**

IV. Discussion

In India, CHIKV was first recorded in West Bengal in 1963–65 along with the dengue outbreak. [3] Thereafter Chikungunya cases were recorded in different states of India till 1973 [11] after which the virus disappeared from India [12] and remained quiescent for almost three decades. In 2005–2006, CHIKV was again reported from many states of India including West Bengal after a gap of 32 yrs. [13, 14] West Bengal is an endemic zone of DENV and several outbreaks have been reported from this region. [15]

A similar study conducted by Harendra S. Chahar et al in 2009 [16] on co-infected patients of Delhi showed that co-infections with CHIKV and DENV occur in areas where these 2 viruses cocirculate and the epidemiology of these viruses is changing with both these viruses are becoming endemic to this region. Thus, in clinically suspected cases it is advisable to test for both viruses in areas where they cocirculate.

Another study by Bhooshan S Gandhi et al in Pune [17] showed similar prevalence of both these viruses in the post-monsoon period similar to our study.However they reported a mortality of 12% as against no death recorded in our study.

The probable reason for the dual infection may be because both the vector mosquitoes Aedes aegypti and Aedes albopictus are abundantly present in West Bengal--Aedes aegypti predominating in the urban areas and Aedes albopictus both in rural and urban areas. [18, 19]

Urbanization, industrialization, and deforestation have resulted in vector shuffling in many areas [18] leading to increased prevalence of both these viruses. Therefore, further epidemiological and virological studies for both the viruses are required not only to formulate control strategies but also reduce morbidity and mortality by instituting early proper treatment.

V. Conclusion

The above study confirmed that there was emergence and spread of *Chikungunya virus* infection in West Bengal, India. Though the disease is associated with low mortality, it leads to high morbidity. The health authorities and the community should therefore, keep a strict vigil for the early diagnosis of the illness.

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