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A SEROEPIDEMIOLOGICAL INSIGHT INTO THE CURRENT STATUS AND RAPID DISSEMINATION OF CHIKUNGUNYA VIRUS INFECTION IN WEST BENGAL – A TWO YEARS RETROSPECTIVE STUDY

Clinical Research

Rajendra Prasad Chatterjee M.Sc- ICMR Virus Unit, ID & BG Hospital campus, GB-4, 1st Floor, 57 Dr. S.C Banerjee Road, Kolkata- 700010, West Bengal, India.

Aroni Chatterjee M.Sc- ICMR Virus Unit, ID & BG Hospital campus, GB-4, 1st Floor, 57 Dr. S.C Banerjee Road, Kolkata- 700010, West Bengal, India.

Nilanjan Chakraborty PhD- ICMR Virus Unit, ID & BG Hospital campus, GB-4, 1st Floor, 57 Dr. S.C Banerjee Road, Kolkata- 700010, West Bengal, India.

Shyamalendu Chatterjee PhD- ICMR Virus Unit, ID & BG Hospital campus, GB-4, 1st Floor, 57 Dr. S.C Banerjee Road, Kolkata- 700010, West Bengal, India. Corresponding Author

ABSTRACT

Chikungunya is currently one of the major public health threats concerning the population in eastern India. Every year, huge numbers of cases of chikungunya-like febrile illness are referred to our institute from different medical colleges and hospitals all around West Bengal for the detection of active chikungunya virus infection. During these two years of study period (2015-2016), a total of 1589 samples were referred to us for this purpose. All the samples were tested for the detection of IgM antibodies by ELISA method followed by RT-PCR method. 498 samples were found to be ELISA positive & 361 samples were RT-PCR positive. This study shows the prevalence status of chikungunya virus infections in the population of West Bengal during the study period, focussing on its rapid resurgence.

KEYWORDS

1. Introduction:-

Chikungunya virus (CHIKV) is an enveloped, positive strand RNA virus belonging to the genus Alphavirus of the Togaviridae famiy. Infection with CHIKV results in chikungunya fever. *Aedes aegypti* and *Aedes albopictus* are the main vectors of CHIKV in Asia and the Indian Ocean region [1]. Following transmission, CHIKV replicates in the skin and spreads to the liver and joints [2]. The incubation period of the virus is 2–4 days [3], followed by a sudden onset of disease with no prodromal phase. The acute phase of the disease lasts for 3–10 days with high fever, rigors, headache, photophobia and a petechial or maculopapular rash [4], but the convalescent phase usually lasts from weeks to months with accompanying joint pain and swelling of joints; sometimes it may last for a year or more [3]. Sporadic cases of neurological complications due to CHIKV have also been reported [5]. CHIKV was first isolated in Tanzania in 1953 [6]. In India, the first CHIKV outbreak was recorded during 1963–1965 in Kolkata (formerly Calcutta) [7] along with an outbreak of dengue fever. In this epidemic, approximately 1, 00,000 cases occurred, with 500 hospitalisations and 200 deaths [8]. Following this epidemic, CHIKV totally disappeared from this region [9], although reports of CHIKV infection from other states of India were observed uptil 1973 [10–12]. The entire isolated virus up to this period belonged to the Asian genotype. A resurgence of CHIKV in India was again reported in 2005–2006, where 1.4 million chikungunya cases were reported from different regions of India [17]. States affected by the outbreaks included Andhra Pradesh [17], Karnataka [17], Maharashtra [17], Orissa [17], Kerala [18], Tamil Nadu [19], Andaman & Nicobar Islands [20], Gujarat [21], Madhya Pradesh [21] and Delhi [21]. The vector responsible for the spread of the disease was identified as *A. albopictus* [22, 23] and the strain responsible was the central/East African genotype of CHIKV [24]. This paper reports the dissemination and rapid spread of CHIKV in the state of West Bengal, including Kolkata, during the years 2015 and 2016.

2. Materials and methods:-

Meteorological Data and Geographical Area of Study:

West Bengal happens to be one of the most important state in eastern India and nation's fourth-most populated state, with over 91 million inhabitants with an area of 88,752 km^2. Population density of this state is 1,000/km2 and presently bordered by the south Asian countries like Bangladesh, Nepal and Bhutan. Frequent infiltration of citizens from other countries/states takes place by crossing the border which may facilitate the emergence of new infection. Approximately 7.5% of the total population of the India lives in this state (see http://censusindia.gov.in/). Kolkata (22˚82'N, 88˚80'E) is the capital of West Bengal and seventh largest metropolitan city in area and population in the world. Kolkata experienced the first epidemic of Chikungunya in 1963–1965. The city has an international seaport and international airport. In addition three big railway station (Sealdah, Howrah & Kolkata), among which Sealdah is the busiest in the world. These three railway stations serve as the gateways of the city. The River Ganges partitions the state of West Bengal into two regions; the northern region known as North Bengal and the southern region known as South Bengal. The state consists of 20 districts, of which Darjeeling, Jalpaiguri and Cooch Behar are located in the hilly and cold climatic regions. The main seasons are summer, monsoon, autumn, late autumn and winter. The summer lasts from mid-March to mid-June, with the temperature ranging from 38˚C to 45˚C. The monsoon arrives by the middle of June and lasts up to September. A high humid temperature prevails in these two seasons which is favourable for both viral growth and mosquito breeding. The breteau index of the vector mosquitoes, *Aedes agypti* and *Aedes albopictus* are moderately high in Kolkata during these two seasons which serve as the potential vector for Chikungunya virus infection.

Patient and Clinical samples:

The ICMR Virus Unit, Kolkata functions as an Apex Referral Laboratory for the detection of Chikungunya virus infection, mostly in the eastern part of India. Blood samples from clinically suspected Chikungunya cases along with a short history of illness and duly signed consent form of patients are mostly referred to the ICMR Virus Unit, Kolkata, from different medical colleges as well as different hospitals and private practitioners for the detection of Chikungunya virus infection.

Patients Inclusion Criteria:

The inclusion criteria for this study were mainly with the patients who were clinically diagnosed as Chikungunya infection having moderate to high fever (104˚F), back pain, headache, myalgia, joint pain, fatigue, arthralgia, maculopapular rash, oedema, petechiae and gingivorrhagia. In this study we considered at least two such symptoms in addition to febrile illness. Patients with any form of bacterial or fungal infection were excluded from the referring institution.

Sample Collection and Serum Separation:

Approximately 4-5ml blood samples from clinically suspected cases were collected by venous puncture by the health workers and medical technicians. During these two years of study period (2015-2016) a total

of 1589 samples were thus received along with a short history of illness. All the samples were transported to ICMR Virus Unit, Kolkata, maintaining the cold chain for the detection of Chikungunya infection, if any. The sera/serum were separated by centrifugation at 1500 rpm for 10 min at 4°C and kept at -80°C in aliquots until tested.

Serological Detection:

For the purpose of serological detection, all of the serum samples received during the study period were subjected to ELISA concurrently for the detection of IgM antibody to CHIKV following the manufacturer's protocol using commercially available kits from SD Diagnostics Pvt. Ltd. (Alere, Republic of Korea). Further MAC-ELISA kits were received from the National Institute of Virology (NIV) (Pune, Maharashtra, India). These kits follow the IgM antibody-capture ELISA technique using specific antigen for CHIKV. The optical density at 492 nm was measured using an ELISA reader (Titertek Multiskan Plus Model 314; Labsystems, Helsinki, Finland).

Isolation of Virus:

A control strain of CHIKV (GenBank accession no. EF027140.1) was procured from National Institute of Virology and was revived in the C6/36 mosquito cell line in a tissue culture system. The control strain was diluted in 1:100 ratio and was inoculated in a monolayer growth of the C6/36 cell line in 25 cm2 tissue culture flasks (Nunc, Roskilde, Denmark). The suspension was allowed to adsorb for 2 h at 28◦C in an incubator with 5% carbon dioxide. Attempts were also made to isolate the virus from 20 selected ELISA-negative samples from patients with a history of ≤ 2 days of fever and chikungunya-like illness in 12-well tissue culture plates (Nunc). The wells were washed with 1 × PBS (Gibco BRL–Invitrogen, Grand Island, NY, USA) and then Eagle's minimal essential medium (Gibco BRL) was added supplemented with 10% fetal bovine serum (Gibco BRL) andpenicillin – streptomycin-neomycin antibiotic mixture (Pen-Strep-Neo; HiMedia Laboratories Pvt. Ltd., IN) and was incubated under the same condition in an incubator. Wells were observed regularly for the appearance of cytopathic effect (CPE) up to 10 days. On appearance of the CPE, the tissue culture fluid was centrifuged at 1000 × g for 10 min at 4◦C to remove cell debris. The supernatant of the tissue culture fluid (STF) was used for further studies.

Molecular Detection:

The STF of those samples that produced CPE were selected for RT-PCR along with the STF of the control strain. RNA was extracted from STF using a QIAamp RNA Viral Kit (QIAGEN Inc., Valencia, CA, USA) according to the manufacturer's protocol. RT-PCR was carried out with a QIAGEN One Step RT-PCR Kit using the primer pair CNP1F 5' (GAA ATT GAT CCC GAC TCA ACC ATC C-3') and CNP1R 5' (CCT TTA ATC GCC TGG TGG TAT AGC-3') 26 and 51 of total RNA. RT-PCR was performed with reverse transcription (50 ◦ C for 20 min) followed by inactivation of the reverse transcriptase enzyme (95 ◦C for 15 min) and 35 cycles of PCR, which included denaturation at 95 ◦C for 30 s, annealing at 55 ◦C for 32 s and extension at 72 ◦C for 45 s. A final extension step was carried out at 72 ◦C for 2 min. PCR products were analysed by running in a 1.5% agarose gel, stained with ethidium bromide.

Statistical Analysis:

SPSS 16 (SPSS, Inc., Chicago, IL, USA) was used for data analysis. Means, standard deviation (SD) and frequencies (percentages) of the socio-demographical and clinical characteristics were calculated for CHIKV infections. The independent sample t-test and Pearson's chi-square were used for comparison of quantitative variables and chi-square test for qualitative variables. Univariate and multivariate analysis were used to find out the association between the characteristics of subjects and CHIKV seropositivity or active infection. Using the Enter method, odds ratios (OR) as well as 95% confidence intervals (CI) were calculated by the multivariate analysis. P value of <0.05 was considered statistically significant.

3. Results:-

Socio-demographic features associated with Chikungunya virus infection among the infected study population:

A total of 1589 patients were enrolled for this study. The study comprised of patients from all the socioeconomic classes with 41.9 % patients belonged to the lowest monthly income group (<5000INR/ month). 60.4% patients belonged to the urban locality and 61.2 % patients were employed. 45.8% patients were illiterate and 67.3% patients lived in extremely unhygienic conditions. A detailed analysis has been put forwarded in **Table 1.**

Chikungunya seroprevalence among the studied population:

Of the 1589 patients enrolled, 498 (31.3%) were seropositive for chikungunya virus immunoglobulin M (IgM), while chikungunya virus ribonucleic acid (RNA) was detected in 361(22.7%) (**Table 1**). Those in the age group of 1-10 years had the highest IgM seropositive rate (50.3%), followed by 35.4 % in the age group 11-20 years. IgM seroprevalence was higher in women (33.3%), in patients from urban locality (39.2%) and those living under unhygienic conditions (34.6%). Patients with no educational exposure were found to be affected at a higher rate (36.1%) than others. Table 1 presents the detailed prevalence pattern of CHIKV with relation to various parameters.

Probable risk factors associated with CHIKV infections:

Multivariate analysis was used for CHIKV IgM and RT-PCR seropositive groups as dependent variable and socio-demographic factors as independent variables. P value < 0.05 was considered statistically significant. We therefore determined if age, gender, socio-economic status, occupation, literacy, residing locality and hygiene had any significance risk to predict the prevalence of CHIKV infections. Age, house hold income, locality of residence and household condition were found to be significant risk factors for CHIKV infection (Table 1).

Table 1- Multivariate logistic regression analysis of socio-demographic features among patients associated with Chikungunya virus infection

Parameters	Total Sample Tested (1589)	Total CHIKV Positive to		CHIKV % Positivity		Odd Ratio (95% CI)	P value
		IgM (498)	PCR (361)	IgM 31.3%	PCR 22.7%		
Age						1.98(0.81-2.6)	0.02
1 -10	264	133	115	50.3	43.5		
11-20	305	108	53	35.4	17.3		
21-30	311	101	57	32.4	18.3		
31-40	299	93	61	31.1	20.4		
41-50	212	31	44	14.6	20.7		
51+	198	32	31	16.1	15.6		
Gender						0.88(0.93-2.6)	0.2
Male	845	250	183	29.5	21.6		
Female	744	248	178	33.3	23.9		
Occupation						2.55(1.3-3.5)	0.15
Employed	973	285	199	29.2	20.4		
Unemployed	616	213	162	34.5	26.2		
Locality						0.76(0.58-1.59)	0.01
Urban	961	377	256	39.2	26.6		
Rural	628	121	105	19.2	16.7		
Monthly Income						3.2(1.99-5.21)	0.002
5000	665	237	177	35.6	26.6		
5001-10000	603	179	138	29.6	22.8		
10000	321	82	46	25.5	14.3		

Educational Status						0.21(0.79-2.2)	0.45
Illiterate	728	263	204	36.1	28.0		
Primary & Secondary	547	164	121	29.9	22.1		
Graduate & above	314	71	36	22.6	11.4		
Household Condition						1.43(0.88-2.1)	0.001
Hygenic	519	127	96	24.4	18.4		
Unhygenic	1070	371	265	34.6	24.7		

During both the years the maximum number of cases was reported during the months of July to November which corroborates with the warm, rainy season in West Bengal, an ideal environment for the growth of mosquitoes and spread of vector borne diseases (**Figure 1A**). The vector usually breeds in the stagnant water and hence the majority of population are directly exposed to the vector during the rainy season. The major cases were observed from the districts of Kolkata, Howrah, North 24 parganas, South 24 parganas, East Medinipur and West Medinipur (**Figure 1B**). The highest numbers of seropositive cases were observed from districts in and around Kolkata. The reason may be due to the fact that these are sea, air and railway connected and bordered districts with major chances of immigration carrying infected individuals. In this study, males and females were found almost equally affected in almost all the cases.

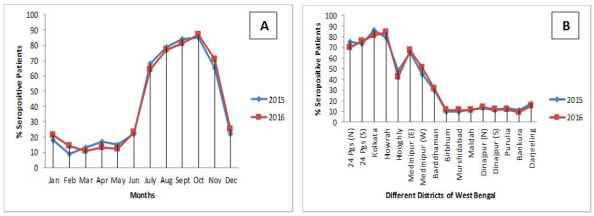

Figure 1- Distribution of seropositive CHIKV cases; A- According to the different months, B- According to the different districts of West Bengal.

4. Discussion:-

The sudden escalating number of Chikungunya cases is creating a wave of panic among the people of West Bengal. At least a few lakh people from the different districts of West Bengal have suffered from Chikungunya and other arbo-viral infections over the past 1 year [25]. But the state health authorities still seems to be unprepared for combating this mosquito menace. About 200-300 cases of Chikungunya infection have been reported from different areas of north and north-east kolkata in the year 2016 only [26]. Thousands may have been affected by Chikungunya in other districts, though only a handful of them have been admitted to the hospitals [27, 28]. This study was aimed to show the current status of Chikungunya infection among the masses in West Bengal. Of the 1589 cases received in two years, 498 (31.3%) were found to be CHIKV IgM positive whereas 361 (22.7%) were confirmed to be PCR positive. Ninety-five percentage of the patients reported of persistent joint pain, arthralgia and physical disabilities. In this study we have also discussed the potential risk factors corroborating with active chikungunya infection among the affected population. While health officials are tight lipped about rising number of cases and are dismissing new cases as resurfacing of the old ones, truly the toll of Chikungunya in West Bengal is mostly obscure. This study merely represents the tips of the ice berg. In depth year round study is immensely required to identify all the cases to evaluate the disease burden in this state. There is an urgent need to spread the awareness about Chikungunya and other mosquito-borne diseases as well as mosquito eradication, among residents to encourage community participation and in order to curb the further spread of disease.

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Conflict of interest

There exists no conflict of interest among the authors.

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