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SEROPOSITIVITY OF CHIKUNGUNYA, CO-EXISTENCE OF CHIKUNGUNYA VIRUS WITH DENGUE VIRUS: A STUDY AT A TERTIARY CARE HOSPITAL, UDAIPUR, RAJASTHAN

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ABSTRACT

Introduction: Diseases due to Arboviruses are one of the major public health problems and a major constituent of emerging infectious diseases worldwide. Out of many Arboviruses, Chikungunya virus and Dengue virus are the two most rapidly spreading and important for medical health. Both the diseases have some common signs and symptoms. **Objectives**: To study the Seropositivity of Chikungunya virus and observation of Co-existence of Chikungunya virus with Dengue virus.**Materials and Methods:** 355 serum samples from patients with febrile illness attending to the inpatient and outpatient department were processed by Chikungunya IgM Capture ELISA and Dengue IgM Capture ELISA. **Results:** Of 355 serum samples, febrile illness was higher in males (54.64%) than in females (45.36%), with male to female ratio 1.2:1. Most affected age group from febrile illness belongs to 21-30 years (36.34%), followed by age group 31-40 (22.82%). 3 (0.84%) were sero-positive, 17 (4.78%) were equivocal and 335 (94.37%) were negative for Chikungunya virus. Of the affected cases with Chikungunya 66.66% were males and 33.34% were females. All the affected cases were belonging to age group 31-40 years. Co-existence of CHIK V and DEN V was found in only 0.28% samples.**Conclusion:** The present study highlights the fact that Chikungunya virus infection is an important cause of febrile illness and emphasizes the need of continuous surveillance for Chikungunya virus disease using multiple diagnostic tests. In the present study Chikungunya Seropositivity is less as compared to other studies whereas Co-existence of Chikungunya with Dengue virus (0.28%) suggests possibilities of both arboviruses in this region.

Keywords: Chikungunya virus, Dengue virus, ELISA.

INTRODUCTION:

Chikungunya and Dengue both viruses are enveloped, RNA Arboviruses, spread by the bite of Aedes aegypti and Aedes albopictus. Chikungunya virus belongs to the genus Alphavirus and family Togaviridae. The name chikungunya is derived from the Makonde word which means “that which bends

up" describing the stooped posture due to arthritic features of the disease. **(1)**

Chikungunya fever was first reported in 1952 from Makonde plateaus, along the borders between Tanzania and Mozambique. **(2, 3)** Chikungunya virus was first isolated by Ross in 1953 during an epidemic in Newala district of Tanzania. **(4)** Dengue virus is belongs to the genus Flavivirus and family Flaviviridae. The name Dengue is derived from the Swahili word 'Ki denga pepo', which means sudden seizure by the demon. **(5)** Following the Philadelphia epidemic in 1780, it was called as the 'Break bone fever' or 'Bone crush disease' by Benjamin Rush. **(6)** The first recognized dengue epidemic occurred almost simultaneously in Asia, Africa and North America in the 1780. The first confirmed case reported from 1789. Both viruses share common clinical symptoms include sudden onset of crippling arthralgia accompanied with fever, chills, headache , nausea, vomiting, low back pain and rashes lasting for a periods of 1-7 day. The incubation period is usually 2-3 days. The acute phase lasts for 2-3 day and may remit for 1-2 days after a gap of 4-10 days resulting in a "saddle back" fever curve.

Co-existence of chikungunya virus and Dengue virus- Due to common vector and similar clinical symptoms these viruses Co-circulate in many areas. The first case report of chikungunya and dengue co-infection confirmed by molecular assays was from Sri Lanka. **(7)** In 1967 co-infection with dengue and chikungunya virus were reported from Kolkata. CHIK V and DEN V are co-circulating in India and Southeast Asia. **(8)** Circulation of chikungunya virus is seen in dengue epidemic area. The clinical manifestation of CHIKV infection mimic DENV infection in these areas and CHIKV infections are misdiagnosed as DENV infection. Serological tests detect anti-CHIK and anti-DEN antibodies using immunochromatographic Rapid card test, IgM capture enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescent method, hemagglutination inhibition, neutralization techniques.**(9,10,11)** Molecular diagnosis of chikungunya and dengue by Polymerase chain reaction (PCR), RT-PCR and Reverse transcription loop mediated isothermal amplification (RT-LAMP).

MATERIALS AND METHODS

This study was conducted in months of September, October and November of 2014 and 2015, in the Serology Laboratory, Department of Microbiology, R.N.T. Medical College, Udaipur, and Rajasthan. During the study period a total of 355 samples collected from patients suffering from fever, joint pain, headache and rashes, who presented in the Inpatient and Outpatient Departments of M.B. Govt. Hospital and Pannadhay Hospital, Udaipur, Rajasthan.

Serum sampling- About 3-5 ml of whole blood collected into the collection tube without anticoagulant, by vein puncture, leave to settle for 30 minutes for blood coagulation and then centrifuged blood to get serum specimen of supernatant.

Test performed – The samples were tested for CHIK and DEN IgM antibody using IgM antibody Capture ELISA kit produced by NIV (Arbovirus Diagnostic NIV, Pune, India). The sensitivity and specificity for the CHIK IgM antibody capture ELISA is 95% and 97.22% respectively, and for Dengue IgM antibody

capture ELISA is 98.53% and 98.84% respectively. The tests were carried out following the manufacturer instruction.

Principle of IgM Capture ELISA: IgM antibody in the patient`s serum are capture by anti human IgM (μ chain specific) that are coated on to the solid surface (wells). In the next step, CHIK/DEN antigen is added, which binds to capture IgM, if the IgM and antigen are homologous. Unbound antigen is removed during the washing step. In the subsequent steps Biotinylated anti-CHIK/anti-DEN monoclonal antibody is added followed by Avidin-Histidin rich protein (HRP). Subsequently, substrate\ chromogen (TMB/H_2O_2) is added and monitored for development of colour. The reaction is stopped by 1N H_2SO_4. The intensity of colour/ optical density (OD) is monitored at 450nm. OD values are directly proportional to the amount of CHIK virus specific IgM antibodies present in the serum sample. The sample considered positive for IgM antibody if the OD of the sample exceeds OD of negative control by a factor 4.0 (sample OD= negative OD×4.0). Both positive and negative controls used to validate the test.

RESULTS

During the study period 355 serum samples were processed from IPD and OPD patients. Of these 194 (54.64%) were males and 161 (45.36%) were females. Majority of the cases were from the age group of 21-30 years (36.34%) followed by age group 31-40 years (22.82%). 3 (0.85%) were positive, 17 (4.78%) were equivocal and 335 (94.37%) were negative for CHIK virus. (Table 1) Of the total number of affected cases by CHIK virus, 33.34% were females and 66.66% males. Affected cases were from the age group of 31-40 years. Out of 355 serum samples, one (0.28%) was positive for both Chikungunya virus and Dengue virus and 2 (0.56%) were equivocal for both the viruses. Co-existence of CHIK V with DEN V was found in 0.28% samples.

DISCUSSION

Area specific monitoring studies which are aimed to gain knowledge about the type of infectious agents which are responsible for the infection, helps the clinicians to choose the right empirical treatment. This is important not only to provide an appropriate therapy, but also for the prevention of the infection.

In this study a total of 355 serum samples were tested for Chikungunya virus by CHIK V IgM Capture ELISA. Of the total samples were tested, only 3 (0.85%) were found to be positive for CHIK V. While Pratima Roy et al **(12)** (2008-09) reported 25.37% in Karnataka, Jaipur (Rajasthan) and New Delhi, Tanmay Mahapatra **(13)** (2010) reported 71% in Delhi and Sudharsanam M Balasubramanian et al **(14)** (2006) reported 22.3% in Chennai, Tamil Nadu. (Table 2) In present study there is significant difference in findings, because previous studies were carried out at the time of chikungunya epidemic. In present situation little number of Chikungunya Seropositivity was present in the population, in spite of fact there is no epidemic. Hence this study gives about local endemically present Chikungunya Seropositivity.

In this study Males (66.66%) were affected more than Females (33.34%), while Sudharshanam M et al **(14)** (2006), reported that females (57.30%) affected more

than males (42.70%). The age group 31-40 years was mostly affected in this study; these findings were much similar to the studies carried out by Sudharshanam M et al **(14)**, Tanmay Mahapatra **(13)** Out of these only 1 (0.28%) was sero-positive for both CHIK V and DEN V and 2 (0.56%) were found equivocal for both the viruses, while Carey DE et al **(15)** (2006) reported (3%), Yergolkar PN et al **(16)** (2006)reported (1.7%) from three states of India during 2006 epidemic, Indrany Mohanty et al **(17)** (2011-2012) reported (1.15%), in Berhampur, Orissa, N Shaikh et al **(18)** (2010-2013) reported (8.11%), in Karnataka and Vikaram Londhey et al **(19)** (2010-2015) reported (6.7%), Mumbai. (Table 3)

CONCLUSION

The present study highlights the fact that Chikungunya virus infection is an important cause of febrile illness and emphasizes the need of continuous surveillance for Chikungunya virus disease using multiple diagnostic tests. In Indian setting, low socio-economic conditions; overcrowding, poor sanitary conditions facilitated by the presence of *Aedes* vector species contribute to the spread of CHIK V. Therefore screening of CHIK V, DEN V and other arboviruses is necessary, because though the clinical features are similar the outcomes may vary. In this Co-existence of CHIK V with DEN V is 0.28%. Co-existence suggest that epidemiology of these viruses is changing in this region. Although in clinically suspected cases of dengue fever or chikungunya fever, it is advisable to keep in mind possibilities of both the arboviral diseases in the region.

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Table 1- Sero-positivity of Chikungunya virus by CHIK IgM Capture ELISA

Total No. of samples 355			
S. No.	**CHIK V IgM ELISA**	**Number of samples**	**Percentage (%)**
1	Positive	3	0.85
2	Equivocal	17	4.78
3	Negative	335	94.37

TABLE 2 - Comparison of Sero-positivity of Chikungunya Virus with Other Studies

S. No.	Studies	Year	Seropositivity of CHIK V
1	Sudharshanam M et al	2006	22.30%
2	Pratima Roy et al	2008-09	25.37%
3	Tanmay Mahapatra	2010	71%
4	Present Study	2014-2015	0.85%

TABLE 3- Comparison of Co-existence of Chikungunya virus with Dengue virus with Other Studies

S. No.	Studies	Year	Co-existence of CHIK V with DEN V
1	Carey DE et al	2006	3%
2	Yergolkar PN et al	2006	1.73%
3	Indrani Mohanty et al	2011-2012	1.15%
4	N Shaikh et al	2010-2013	8.11%
5	Vikaram Londhey et al	2010-2015	6.70%
6	Present study	2014-2015	0.28%