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Original Research Paper

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SEROPREVALANCE OF CHIKUNGUNYA IN DENGUE NEGATIVE SAMPLES AT A TERTIARY CARE HOSPITAL – A HIDDEN BURDEN

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ABSTRACT Chikungunya fever is an arthropod-borne viral disease. Chikungunya and Dengue have a common vector and shows similar clinical presentation. Even though Chikungunya virus infection is self-limiting and less lethal, more than 30% of affected individuals will have post chikungunya chronic polyarthralgia which will affect the patient's quality of life. Reverse transcriptase polymerase chain reaction has high sensitivity and specificity in diagnosis but Enzyme linked immunosorbent assay looks more economical and it provides diagnostic information as well as insights into immune response to CHIKV infection. This is a prospective study conducted from July 2022 to December 2022 in a tertiary care center. Blood samples were collected from the patients presented with acute febrile illness. The samples which were negative for dengue by serological methods were subjected to IgM capture ELISA for chikungunya. Total of 773 samples were negative for dengue fever in patients with acute febrile illness during the study period. These samples were subjected to IgM capture ELISA for Chikungunya. Out of 773 samples 75 samples were positive for chikungunya virus. Total prevalence in the study was 9.7%. Our Study has shown equal sex distribution. Majority of the patients are from urban places. More positive cases were reported in the month of September. Our study has shown that Chikungunya fever is the most important viral illness to be considered in cases of acute febrile illness especially in endemic areas like India, where it goes undetected because evaluation will be more towards dengue fever rather than CHIKV infection. Timely and appropriate management can prevent the complications due to chikungunya and can assist in the prediction and control of outbreaks.

KEYWORDS : Chikungunya virus, Capture ELISA,

INTRODUCTION

Chikungunya fever is a mosquito born acute febrile illness caused by a RNA virus belongs to family Togaviridae and genus Alphavirus [1]. CHIKF first described in 1952 in south eastern Tanzania. First outbreak in India was reported in 1963 from Kolkata and then reported from various states like West Bengal, Andhra Pradesh, Maharashtra [2]. CHIKV is a re-emerging pathogen usually causes mild self-limiting illness characterized by fever, myalgia, arthralgia and skin rash. Severe arthralgia or joint pain is the main manifestation. The joint pain is symmetrical and polyarticular involving both small and large joints [3]. Even though the mortality and morbidity in CHIKF is low when compared with other viral illnesses like dengue fever, a significant proportion of the patients may suffer from "post chikungunya polyarthralgia" after 3 months of disease onset. This condition persists for years and characterized by debilitating joint pains which will affect the quality of life of the patients [4]. The acute phase of the illness sometimes characterized by maculopapular skin rash, edematous itchy skin affecting mainly face and trunk. Atypical symptoms are rare and includes neurological and cardiovascular manifestations like encephalitis, myelitis, peripheral neuropathy, myelopathy [3]. Even though viral culture is the gold standard method for isolation of virus, it is not done routinely as it is time consuming and laborious. Various molecular methods are available like conventional PCR, real time RT-PCR etc which are useful in acute phase of illness (<5days). Other than serum and plasma, CSF is also used for PCR. Serological methods can yield diagnosis as well as provides insight into immune response to infection.

Commercial and in-house Enzyme Linked Immunosorbent Assays (ELISA) are available and the latter has more sensitivity [5]. IgM capture ELISA is commonly used. IgM antibodies peak between 5-20 days after infection and persist for up to 11 to 14 months. Hence the results are more accurate if the samples are collected after 5 days after the onset of fever. Rapid diagnostic tests for anti CHIKV IgM and IgG detection have been developed. But they have low sensitivity and specificity. CSF can be used for ELISA in patients with neurological manifestations with 80% sensitivity and 87% specificity. Methods for antigen detection are under development. As dengue and chikungunya possess similar clinical features and there is high mortality rate associated with dengue when compared with CHIKF, evaluation will be more towards dengue fever where CHIKF may be undiagnosed. This is common especially in dengue endemic areas like India.

METHODS:

The present study is a prospective study conducted at Osmania general hospital, Hyderabad from July 2022 to December 2022. The serum samples were collected from patients presented with acute febrile illness. Those samples which are negative for dengue, were subjected to chikungunya IgM capture ELISA. The ELISA kit was provided by National Institute of Virology, Pune. About 5-10 ml of blood is collected from the patients preferably after 5 days of fever onset. Serum was separated from all the samples and IgM capture ELISA for DENV carried out according to instructions given in kit insert provided by manufacturer. Those samples which were negative for dengue IgM were subjected to capture ELISA for anti-CHIKV IgM antibodies. IgM antibodies in the patient's serum are captured by anti-human IgM that are coated on to wells. In the next step, CHIK antigen is added, which binds to captured IgM, if the IgM and antigen are homologous. Unbound antigen is removed during the washing step. In the subsequent steps Biotinylated anti-CHIK monoclonal antibody is added followed by Avidin-Histidine rich protein. Subsequently, chromogen substrate 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 450 nm. Optical density (OD) values are directly proportional to the amount of CHIK virus-specific IgM antibodies present in the sample. The sample was considered positive for IgM antibody if the OD of the sample exceeds OD of negative control by a factor 3.0

(sample OD ≥ negative OD × 3.0). Both positive and negative controls were used to validate the test. As these samples were collected and tested during monsoon and post monsoon when prevalence will be more and patient details were not disclosed to maintain the privacy, ethical approval is not required.

RESULTS:

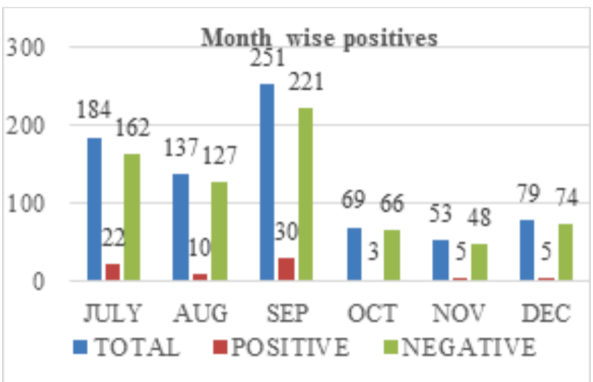
Out of 773 samples tested, 75 (9.7%) were positive for anti-chikungunya IgM. Out of 75 positives 38 (50.6%) were females and 37 (49.4%) were males. Maximum affected individuals are young adults belong to 21-30 years age group. Highest number of positive cases were recorded in the month of September. Most of the patients (81.4%) are from urban areas. All the patients (100%) had presented with fever. Majority of the patients had myalgia, joint pains and weakness. Rash is seen only in two patients. Some patients presented with abdominal pain, diarrhea and vomiting. Two patients were found dead out of 75 positive cases after a month, may be due to post Chikungunya complications but reasons not clearly known. Both were females, one is 26 years old and other patient is 45 years old. The latter patient has diabetes, hypertension and chronic kidney disease.

Table 1: Socio demographic characteristics of the patients:

Characteristics	Number (n=75)	Percentage (%)
Gender		
Male	37	49.4%
Female	38	50.6%
Age group (in years)		
<10	3	4%
11-20	15	20%
21-30	16	21.4%
31-40	10	13.4%
41-50	14	18.6%
51-60	13	17.3%
>60	4	5.3%
Region		
Urban	61	81.4%
Rural	14	18.6%

Table 2: Clinical Symptoms

Clinical symptoms	Number of cases (n=75)	Percentage
Fever	75	100%
Malaise and weakness	60	80%
Joint pains	52	69.3%
Headache	31	41.3%
Diarrhoea	12	16%
Abdominal pain	11	14.6%
vomitting	8	10.6%
Rash	2	2.6%



DISCUSSION:

Chikungunya fever and dengue fever are the two globally important arboviral diseases. They are transmitted by Aedes mosquitos and possess similar clinical features. Most of the times, as the dengue fever is more lethal than chikungunya fever, evaluation will be more towards dengue where CHIKF goes undetected especially in endemic areas like India. The main objective of this study is to establish a diagnosis for acute febrile illness other than dengue. As in a large government set up like our institution where resource limitation is the major problem this study was undertaken only on dengue seronegative samples. Our study has showed 9.7% of prevalence. In a study by Prafulla Dutta et.al (2019) the total positivity rate is 11.2%. Previous serosurveys from different geographical settings in India have reported CHIKV seroprevalence ranging from 2·9% to 68 %. Many studies showed higher prevalence from different parts of India, and that can be due to higher sample size. In other studies which showed higher prevalence the serological tests were done in all the patients who presented with acute febrile illness. This might be one of the important reasons for less prevalence in the current study where IgM capture ELISA was done only in dengue seronegative cases. Both males (49.4%) and females (50.6%) were equally affected in the current study. In a study conducted by Solanki Manoj et.al (2018) males were 30(44.78%) and females were 37(55.22%). In a study [6] positive cases were slightly higher in males with male to female ratio of 1:0.75 as males are associated with more activity during day time and have chances of increased mosquito bites. In studies conducted by Mahesh Kumar S [7] in 2018 and Singh J et.al study [1] majority were in age group of 20 to 30 years and fever is the common symptom in all the patients. The least affected are extremes of age groups because of reduced outdoor activities and more protected lifestyle mainly in pediatric age groups. Like in many other previous studies highest cases were reported in monsoon season in our study this could be due to the fact that monsoon and post monsoon seasons are the breeding periods for Aedes mosquito. According to a study done by Piyush Joshi et.al, 2020 [8] CHIKF cases in rural and urban areas revealed higher positivity in urban (56.07%) areas than rural (43.92%) areas. In our study more cases are reported from urban areas (81.4%). In a study done by Thiberville et.al, 2013 symptoms such as diarrhoea, abdominal pain, nausea and vomiting are seen in 15-47%. In our study diarrhoea, abdominal pain and vomiting are reported in 16%, 14.6% and 10.6% respectively. Death is uncommon in CHIKF, but according to a study conducted by de Morais Alves Barbosa Oliveira R et.al, [9] the factors related with deaths were chronic kidney disease and previous heart disease, presence of fever, abdominal pain, apathy, dyspnea and arthritis and laboratory findings such as leukocytosis, leukopenia, thrombocytopenia, neutropenia and lymphopenia. In our study 2 (2.6%) cases were found to be dead after a month post CHIK fever for reasons not clearly known and could not be evaluated due to lack of proper follow up. Both were females, one was 26 years old and other was 45 years old. Second patient had comorbidities like diabetes, hypertension and had chronic kidney disease.

CONCLUSIONS

Chikungunya outbreak is a major public health problem in India. Even though CHIKF is mild self-limiting viral illness, the clinical sequalae with debilitating joint pains affects the quality of life of an individual. Hence it is very important to take the preventive measures to cut down the transmission mainly during monsoon and post monsoon seasons. This study has also concluded that CHIKF is an important viral infection to be considered in patients presenting with acute febrile illness. As there are no licensed vaccines and antivirals are available, it needs to be focused on prior surveillance and implementation of preventive measures.

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