Co-circulation of dengue virus serotypes with chikungunya virus in Madhya Pradesh, central India

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ABSTRACT

Background: Dengue and chikungunya present with very similar signs and symptoms in the initial stage of illness and so it is difficult to distinguish them clinically. Both are transmitted by Aedes aegypti and Aedes albopictus mosquitoes. This study was conducted with the aim to explore the co-circulation of dengue and chikungunya viruses in central India.

Materials and methods: Samples from suspected dengue cases were subjected to dengue immunoglobulin M (IgM) enzyme-linked immunosorbent assay (ELISA) and dengue-negative samples were tested with chikungunya-specific IgM ELISA. The samples collected in acute phase of illness were tested by nested reverse transcription polymerase chain reaction (nRT-PCR). Chikungunya virus (CHIKV) sequences were analysed to determine their genotype.

Results: Of 138 samples screened for dengue, 21 (15.2%) were positive, and of 119 samples screened for chikungunya, 13 (10.9%) were positive. Dengue viruses 1 and 4 were found co-circulating with chikungunya virus in Jabalpur, central India. The chikungunya virus detected belonged to the East Central South African genotype.

Conclusion: Accurate and timely diagnosis would help in patient management and use of resources. It is advocated to simultaneously test samples for these two diseases in endemic areas. This will also aid in understanding the epidemiology of chikungunya.

Key words: Central India, chikungunya, co-circulation, dengue

INTRODUCTION

Arthropod-borne viral infections cause major disease burden in tropical and subtropical countries worldwide. The incidence of dengue has increased more than 30-fold in the last 50 years.¹ It is estimated that about 100 million dengue cases and over 390 million infections occur worldwide annually.¹,² India contributes about 34% of these cases.³ Four antigenically related serotypes of dengue virus (DENV), with different genotypes, are known to be circulating in India.³ All four serotypes can cause simple febrile illness (DF), which may lead to more complex clinical outcomes such as dengue hemorrhagic fever (DHF), dengue shock syndrome (DSS) or death.³ Chikungunya re-emerged in 2005, after a gap of 32 years, with about 1.4 million cases, and continues to circulate in India,⁴ with a predominance of the East Central South African genotype of virus.³,⁴ Dengue-confirmed and chikungunya-suspected cases have been reported from this part of the country in the recent past.⁴ Infection with the chikungunya virus is generally self-limiting but in a few cases it may cause severe incapacitating arthralgia in small joints, lasting up to 6 months or more.⁴

DENV and CHIKV are both transmitted by Aedes aegypti and Aedes albopictus mosquitoes. In the initial stage of illness, both diseases present with a similar set of clinical symptoms, such as sudden onset of high-grade fever, muscle and joint pain, headache, nausea and vomiting, and rash,⁴ making differential diagnosis difficult clinically. Chikungunya
has low/no mortality, whereas for dengue, mortality may go up to 3–5% in outbreak situations and serious cases, though early referral and good management can reduce this. In the absence of any licensed vaccine for either of these diseases, timely intervention by prompt accurate diagnosis, along with mosquito control, are the best tools for controlling or avoiding outbreaks in endemic areas.

Chikungunya can spread with ease and causes a high percentage of clinical cases with a very high attack rate in an immunologically naive population. However, chikungunya is overshadowed by dengue in outbreak situations and in dengue-endemic areas.

Diagnosis of chikungunya is equally important, as it would help to estimate the disease burden and, in turn, help with designing intervention strategies. Co-circulation and coinfections of these two viruses are reported. This study sought to confirm the co-circulation of the two viruses in central India, using serological and molecular tools.

MATERIALS AND METHODS

The virology laboratory of the Regional Medical Research Centre for Tribals (RMRCT), Jabalpur is recognized as the Apex Referral Laboratory by the National Vector Borne Disease Control Program (NVBDCP) for Madhya Pradesh and Chhattisgarh.

Ethical approval

Samples were referred to this virology laboratory for diagnosis of diseases and thus considered as part of the public health response; nonetheless, the project “Establishment of Grade II Virology Laboratory” had ethical clearance from the institution ethics committee of RMRCT, Jabalpur, India.

Samples and testing

This year-round (April 2011 to March 2012) study was conducted in Madhya Pradesh. Blood samples of patients reporting to health-care units of Jabalpur and the adjoining 14 districts, with symptoms of dengue and chikungunya, as defined by NVBDCP, were collected by clinicians and referred for diagnosis to the virology laboratory of RMRCT, along with clinical and demographic information in the World Health Organization’s (WHO’s) predesigned format.

Upon receipt, serum was separated by brief centrifugation at 4°C and was preferably tested on the same day by immunoglobulin M (IgM) enzyme-linked immunosorbent assay (ELISA) or nested reverse transcription polymerase chain reaction (nRT-PCR).

The testing sequence for the samples is shown in Figure 1. All the samples were tested by dengue-IgM-specific ELISA; DENV-ELISA-negative samples were tested by chikungunya IgM ELISA, using kits manufactured by the National Institute for Biological Standards and Control, London.

Figure 1: Flowchart depicting the methodology of processing the samples

ELISA: enzyme-linked immunosorbent assay; CHIKV: chikungunya virus; DENV: dengue virus; IgM: immunoglobulin M; nRT-PCR: nested reverse transcription polymerase chain reaction
of Virology, Pune, India, as described by manufacturer. A subset of samples, which were collected in the acute phase of illness, were also subjected to DENV nRT-PCR,\textsuperscript{10} for detection of DENV RNA and the DENV serotype. Of these, samples for which the DENV nRT-PCR result was negative were subjected to CHIKV-specific RT-PCR,\textsuperscript{11} with minor modifications. The PCR products were extracted and sequenced as described earlier.\textsuperscript{12} The sequences were analysed for their homologies, using the basic local alignment search tool, and representative sequences were submitted to GenBank. The partial nucleotide sequence of CHIKV envelope gene 1 (E1) was compared with 14 sequences of CHIKV strains from the National Center for Biotechnology Information (NCBI) database from Asia and Africa. Multiple sequence analysis was done using Molecular Evolutionary Genetics Analysis (MEGA), version 5, with application of 1000 bootstrap replicates.\textsuperscript{13}

The data were double-key entered into Microsoft Excel. Single-sample proportion testing and logistic regression were done using SPSS version 20.

**RESULTS**

One hundred and thirty-eight samples from 15 districts of Madhya Pradesh were screened for dengue (see Figure 1). Of these, 63 (46%) patients were from rural areas; 85 (62%) were male and 53 (38%) were female. The results for age, sex and screening tests for diagnosis of dengue and chikungunya are given in Table 1. A total of 15.2% (21/138) were positive for dengue and 10.9% (13/119) were positive for chikungunya. Single-sample testing confirmed that there was no significant difference between these two proportions (\(P = 0.117\)).

Most of the patients, where infection was suspected – 112 (81%) – had fever (100–103°F) for more than 2 days, with associated symptoms such as headache, body ache, joint pain, fatigue and nausea. Hepatomegaly was seen in 18 (13%) cases. The platelet count was available for 42 patients, of whom 26% had thrombocytopenia (platelet count <105/mm\(^3\)). No cases of DHF, DSS or death were noted. Statistical analysis revealed no significant difference in initial symptoms such as fever in confirmed cases of dengue and chikungunya.

Out of 138 samples tested, 21 (15.2\%) were found to be positive for dengue, from four districts, namely Jabalpur (DENV-1 and DENV-4), Katni (DENV-4), Panna and Narsinghpur (DENV-4). Of the 21 dengue-positive cases, 15 were only IgM ELISA positive, two were only RT-PCR positive and four samples were positive on both tests.

The nRT-PCR and sequencing results confirmed that DENV-1 \((n = 1)\) and DENV-4 \((n = 6)\) were circulating in and around Jabalpur, central India. Most of the confirmed cases of dengue and chikungunya were detected in the monsoon and post-monsoon periods.

DENV-1 was first identified in central India and the sequence showed that it was closely related to the DENV identified from New Delhi (JF81509.1). DENV-4 identified in this study had sequence homology with DENV-4 identified in 2010 (GenBank: JF929180).\textsuperscript{12}

Out of 119 dengue-negative samples, 13 (10.9\%) were positive for chikungunya. All chikungunya-positive samples \((n = 13)\) were from Jabalpur district. Six samples were IgM ELISA positive and nine were RT-PCR positive; of these, two samples were positive on both tests. The phylogenetic analysis carried out using nucleotide sequences established that CHIKV from Jabalpur belonged to the East Central South African (ECSA) genotype (see Figure 2), with close sequence homology with isolate from Delhi (GenBank: JN048826).

It was noted that, in the case of dengue, the positivity was higher among males (16/85; 19\%) as compared to females (53/5/3; 9\%), though the difference was not statistically significant. In the case of chikungunya, no such difference was observed. After adjusting for the sex of cases, the positivity of dengue was significantly higher among adults (age 16 years and above) when compared to children (\(\leq 15\) years; odds ratio [OR] = 31.1; 95\% confidence interval [CI] = 6.8 to 142.8; \(P < 0.01\)) but in the case of chikungunya, this difference was not significant (OR = 1.5; 95\% CI = 0.44 to 5.5; see Table 1).

**DISCUSSION**

DENV-3 was detected during an outbreak that occurred at Jabalpur in 1966.\textsuperscript{14} However, dengue and chikungunya remained neglected diseases in this part of country, with very few studies focusing on these infections.\textsuperscript{3,12,15} Because of the emergence of different serotypes and their genotypes, dengue epidemiology is continually changing, posing challenges to clinicians and health authorities. CHIKV re-emerged in 2005,
after a gap of almost 32 years, and made its presence felt in India with more than one million cases. However, after the epidemic, the medical fraternity neglected CHIKV infections, probably because chikungunya is considered to be a disease with mild morbidity and no mortalities. In contrast, dengue is a disease with significant morbidity and mortality and is endemic in the country. As reported earlier, and also seen in this study, the initial symptoms of chikungunya are similar to those of dengue and both are transmitted by same vectors \textit{Aedes aegypti} and \textit{Aedes albopictus}. Probably because of the severity of dengue, both clinicians and public health officials focus on this disease, and samples are sent to laboratories with requests for diagnosis of dengue only. The present study demonstrates that DENV and CHIKV are co-circulating in Jabalpur, and there is no significant difference in the positivity for the two diseases. Although this study has a limited number of samples, it emphasizes the importance of chikungunya diagnosis, as there is no significant difference in the positivity of samples for the two diseases.

Serological tests (ELISA) have demonstrated the presence of dengue and chikungunya from central India in the recent past. With establishment of the Viral Diagnostic Laboratory, it was possible to provide molecular diagnosis of DENV, with identification of the serotype, and to conduct characterization studies on CHIKV, which lead to identification of DENV serotype 1 and CHIKV ECSA genotype (see Figure 2).

It is known that, before 1973, the Asian genotype was circulating in India and was subsequently replaced by the ECSA genotype. This study documents, for the first time, circulation of the ECSA genotype in this part of India. It will be interesting to isolate and further characterize these circulating viruses for better epidemiological understanding.

In an endemic area like India, where multiple DENV serotypes are circulating with CHIKV, the possibility of concurrent infection occurs. An earlier study reported the presence of DENV serotype 4 in central India; detection of another serotype (DENV-1) is alarming. There is some evidence that infection with multiple DENV serotypes, or coinfection with other pathogens, may produce a severe outcome of the disease. An early and accurate diagnosis can help clinicians to decide the course of treatment.

The presence of IgM for DENV and CHIKV can only be detected around the fifth day of infection; the tests such as nonstructural protein (NS1) detection for DENV and RT-PCR for both these viruses to detect virus antigen/RNA should be preferred. As apparent in the present study, although based on a small sample size, the early symptoms cannot clinically distinguish between dengue and chikungunya; thus, we advocate early and simultaneous testing of samples for DENV and CHIKV.

**ACKNOWLEDGEMENTS**

The authors are grateful to the Secretary to the Government of India, Department of Health and Research, Ministry of Health and Family Welfare, and The Director-General, Indian Council of Medical Research, for financial support under the project “Establishment of Grade II Virology Laboratory”. The financial support and the kits provided by the National
Vector Borne Disease Control Programme, New Delhi, India is acknowledged. Help from Dr RK Sharma for statistical analysis and technical help from Virology Laboratory staff of the Regional Medical Research Centre for Tribals is also acknowledged.

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How to cite this article: Barde PV, Shukla MK, Bharti PK, Kori BK, Jatav JK, Singh N. Co-circulation of dengue virus serotypes with chikungunya virus in Madhya Pradesh, central India. WHO South-East Asia J Public Health 2014; 3(1): 36–40.

Source of Support: The study was funded by the Indian Council of Medical Research (ICMR), under ICMR’s Viral Diagnostic Laboratory network project, and the Directorate of National Vector Borne Disease Control Program, New Delhi provided kits for diagnosis of dengue and chikungunya. Conflict of Interest: None declared. Contributorship: PVB and NS designed the study and did literature search and writing. BKK and PVB did data collection, data analysis, data interpretation and writing. JKJ did sample collection, clinical diagnosis and treatment. PVB and MKS did data analysis and interpretation (ELISA, nRT-PCR). MKS and PKB did data analysis and interpretation (sequencing and sequence analysis) and writing. All authors read and agreed upon the manuscript.