Journal of Infection and Public Health xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Infection and Public Health

journal homepage: http://www.elsevier.com/locate/jiph

Hidden burden of chikungunya in North India; A prospective study in a tertiary care centre

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ARTICLE INFO

Article history:
Received 11 May 2017
Received in revised form 12 July 2017
Accepted 9 September 2017

Keywords:
Chikungunya
Dengue haemorrhagic fever
Co-infection
Vector borne diseases
Joint pain

ABSTRACT

Background: Arboviral diseases, such as chikungunya, dengue and now zika represent a public health problem, especially in tropical countries. Epidemiology of chikungunya and dengue is well known, including its social and climatic factors associated, but only few data and reports of chikungunya are available from North India. The clinical differentiation of chikungunya from dengue is no doubt challenging since both diseases can share clinical signs and symptoms leading to potential misdiagnosis of chikungunya in areas where dengue is endemic. The aim of this study was to know the seroprevalence, seasonal trends, clinical presentations of chikungunya and its co-infection with dengue virus.
Methods: This was a prospective study conducted in Varanasi, from January to December 2016. All serum samples were tested for both chikungunya and dengue IgM antibodies by MAC ELISA test.
Results: Total of 186 samples, out of which 108 (58%) samples were total seropositive, 23 (12.37%) samples positive for chikungunya IgM antibodies, 57 (30.65%) samples positive for dengue and 28 (15.05%) samples positive for both chikungunya and dengue. The most affected age group was 20-30 years and males were more affected than females. A seasonal peak for chikungunya and its co-infection with dengue were seen in November.
Conclusion: In India, the seroprevalence of chikungunya is increasing. India is a rapidly developing country where adequate sanitation is required. More aggressive intervention and vigilance by health authorities is needed to decrease vector born diseases.

Introduction

Chikungunya affects humans of all age groups worldwide. Chikungunya virus (CHIKV) which belongs to the genus Alphavirus in the family *Togaviridae* and dengue virus (DENV), belong to the genus Flavivirus in the family *Flaviviridae*, are SS(+)RNA viruses. Both pathogens (chikungunya and dengue virus) are transmitted by the same vector i.e. Aedes species mosquitoes. Therefore, it is a reasonable expectation that the epidemiology of chikungunya and dengue infections is temporally and spatially related. To our knowledge, however, there is not yet study describing seroprevalence of chikungunya from North India in past many years. There are few studies from other parts of India indicating its importance as well as analyzed in detail its epidemiological implications [1,2]. As dengue fever has a high incidence and mortality rate, the symptomatic patients are tested only for dengue virus and in rare cases for chikungunya viral infection because of lack of public awareness and near similar clinical presentation. As a result, chikungunya cases go undiagnosed in dengue endemic regions, and the true burden of the chikungunya viral infection has been missed.

Material and methods

About 5–10 ml of whole blood sample was collected from suspected viral fever patients, after 5 days of fever and transported by the staff of the medical college, district headquarters and other hospitals to the department of microbiology in an ice box maintained at 2–8 °C temperature within 24–48 h. It is a sentinel laboratory for diagnosis of chikungunya and dengue by National Vector Born Disease Control Programme (NVBDCP). All serum samples along with details of the patient and clinical history were received in the department of microbiology, Institute of Medical Sciences (IMS), Banaras Hindu University (BHU) from hospitals of IMS, BHU, heritage institute of medical sciences (HIMS), and various primary health centres (PHC) & community health centres (CHC) under the districts headquarter hospitals of Varanasi, Jaunpur, Chandauli, Sultanpur, Mirzapur & Ghazipur, from the month of January to December 2016. The samples were tested for both dengue and

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http://dx.doi.org/10.1016/j.jiph.2017.09.008

Table 1
Demographic characteristics and district wise distribution of cases.

Characteristics		Samples tested (n = 186)	Positive samples (108)	CHIKV (23)	DENV (57)	CHIKV + DENV (28)
Age group (Years)		43	6	1	3	2
	<20					
	20–30	65	39	5	26	8
	31–40	27	23	6	13	4
	41–50	33	28	8	10	10
		18	12	3	5	4
	>50					
Sex	Male	112	83	17	45	21
	Female	74	25	6	12	7
Residence	Rural	123	92	19	48	25
	Urban	63	16	4	9	3
District	Varanasi	96 (51.6%)	61 (56.48%)	13 (56.52%)	29 (50.88%)	19 (67.86%)
	Jaunpur	31 (16.6%)	9 (8.33%)	1 (4.34%)	7 (12.28%)	1 (3.57%)
	Chandauli	49 (26.3%)	36 (33.33%)	9 (39.13)	19 (33.33%)	8 (28.57%)
	Others (Sultanpur Mirzapur, Ghazipur)	10 (5.37%)	2 (1.85%)	0	2 (3.51%)	0
	Total	186	108 (58.06%)	23 (12.37%)	57 (30.65%)	28 (15.05%)

chikungunya IgM antibody using IgM antibody capture ELISA kit produced by National Institute of Virology (Arbovirus Diagnostic NIV, Pune, India). The sensitivity and specificity for chikungunya IgM antibody capture ELISA are 95.00% and 98.00% respectively and for the dengue 98.53% and 98.84%, respectively. The tests were carried out following the manufacturer instructions.

The principle of IgM capture ELISA for DENV & CHIKV

IgM antibodies in the patient's serum are captured by anti-human IgM (μ chain specific) that are coated on to the solid surface (wells). In the next step, DEN/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-DEN/anti-CHIK monoclonal antibody (DEN-B/CHIK-B) is added followed by Avidin-Histidine rich protein (HRP). Subsequently, substrate/chromogen substrate (TMB/H2O2) is added and monitored for development of colour. The reaction is stopped by $1NH_2SO_4$. The intensity of colour/optical density (OD) is monitored at 450 nm. Optical density (OD) values are directly proportional to the amount of DEN/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. In India, National Institute of Virology (NIV), Pune, is a WHO collaborating centre for arboviral diseases. It is engaged in diagnosis, outbreak investigations and preparations of reagents for diagnosis of arboviral infections. It is the only institute in the country that prepares reagents for laboratory diagnosis of DEN/CHIK virus. The enzyme-linked immunosorbent assay (ELISA) test used in this study was developed in-house by NIV, Pune. Laboratory diagnosis depends on the quality of sample and time of collection in the course of illness. In first 5 days, viremia is present and can be confirmed by viral culture, Polymerase chain reaction (PCR) or antigen detection. DEN/CHIK IgM becomes detectable around 5 days of fever and persists for several months and Immunoglobin G (IgG) is present by 10–14 days. Serological diagnosis of CHIK by detecting IgM or IgG seroconversion is widely used because it is cheaper and easier to perform. The sensitivities for diagnosing acute DEN/CHIK virus by IgM detection in 1st week were 4–st week were 4–22% and after one week rose to >80% as compared with PCR.

Statistical analysis

Data were analyzed online by using GraphPad software QuickCalcs version. The results of this study are presented in percentages. Test of significance of the difference between two independent proportions was performed by Fischer's Exact test. Level of significance were as extremely significant [es] (P value < 0.001), very significant [vs] (P value 0.001–0.01), significant [s] (P value 0.01–0.05), not significant [ns] (P value ≥ 0.05).

Results

Out of 186 samples, 108 (58%) samples were positive either for chikungunya, dengue or both. Total 108 positive samples were distributed as 23 (12.37%) cases for chikungunya IgM antibodies and 57 (30.65%) cases positive for dengue whereas only 28 (15.05%) samples were positive for both. Males were predominantly affected as 83 (76.85%). Most common seropositive age group was 20–30 years as 39 (36.11%) cases while 12 (11.11%) cases of chikungynya in group >50 years. The rural population was dominantly affected as most of other vector born diseases. Total seropositivity and chikungunya infection were highest from Varanasi district as 61 (56.48%) and 13 (56.52%) cases followed by chandauli district 36 (33.33%) and 9 (39.13%) cases as represented in Table 1. A seasonal peak for dengue was seen in the month of October while maximum cases of chikungunya and co-infection were seen in November as shown in Fig. 1.

All cases of chikungunya infection had fever, headache, joint pain and fatigue (100%) while no case had retro-orbital pain, diarrhoea, haemorrhagic manifestation, ascites and pleural effusion, on the other hand, common symptomology of dengue included fever 75 (100%), fatigue 61 (100%), fatigue 61 (81.33%), nausea/vomiting 47 (62.66%), headache 43 (62.66%), headache 43 (57.33%) and haemorrhagic manifestation 11 (14.66%) respectively. Headache, joint pain and joint swelling were extremely statically significant (p = 0.0001) for chikungunya, whereas haemorrhagic manifestations were statically significant (p = 0.0284) for dengue infection (Table 2).

Discussion

After a quiescence of about three decades, an outbreak of chikungunya with cases of dengue is being reported from different parts of India. During the emergence of chikungunya virus in the state, it spread in rural and urban areas. On the other hand, dengue virus has four closely related serotypes DENV 1, 2, 3 and 4. Dengue infection has self-limited to life-threatening clinical features. Clinically dengue infection is divided as dengue fever (DF), dengue hemorrhagic fever (DHF), dengue shock syndrome (DSS) and expanded dengue syndrome [3]. Expanded dengue syndrome is new entity describing atypical manifestations of hepatic, gas-

Please cite this article in press as: Dinkar A, et al. Hidden burden of chikungunya in North India; A prospective study in a tertiary care centre. J Infect Public Health (2017), http://dx.doi.org/10.1016/j.jiph.2017.09.008