Original Article

Simultaneous detection of IgM antibodies against dengue and chikungunya: Coinfection or cross-reactivity?

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Abstract

Background: Dengue and chikungunya sharing same mosquito vector are two most important arboviruses circulating in northern India including Delhi and are responsible for frequent outbreaks. Antigen and antibodies detection ELISA kits are the major tool to diagnose these viral illnesses, and are sometimes associated with cross-reactivity, giving a false picture of coinfection, although simultaneous harboring of both the viruses is not uncommon. Various studies have reported coinfection up to 25% from the same region. **Procedure:** This study was conducted in the Department of Microbiology, Maulana Azad Medical College, New Delhi, during the month of September 2016 which included 200 blood samples from clinically suspected cases attending Medicine OPD of associated Lok Nayak Hospital, New Delhi. Diagnosis of dengue and chikungunya was made using NS-1 antigen and IgM MAC ELISA for dengue and IgM MAC ELISA for chikungunya as per manufacturer's instructions. **Results:** Out of 200 suspected cases, 34 (17%) were positive for dengue serology, 77 (38.5%) were positive for chikungunya serology, and 29.9% of positive chikungunya cases were simultaneously affected with dengue. This higher percentage of coinfection might be because of cross-reactivity of the ELISA kits. **Discussion:** India being a hyperendemic region for dengue and chikungunya, frequent outbreaks are quite common. Circulation of both the virus and huge susceptible population are the major causes for frequent outbreaks. Restricting our attention to diagnose one of them is not sufficient, and coinfection further complicates the illness. **Conclusion:** Simultaneous diagnosis of dengue and chikungunya is need of time to diagnose dual infection and prevent complications by starting supportive treatment well in time. Molecular technique if ever possible should be employed whenever the coinfection number is higher than expected to rule out cross-reactivity.

Keywords: Dual infection, IgM antibody, MAC ELISA, NS1 antigen, outbreak

Background

Arthropod transmitted viruses, also known as arboviruses, are global threat especially in tropical and subtropical countries including India. These viruses are transmitted to human through blood sucking insect vectors commonly by mosquitoes and ticks. India has witnessed several outbreaks of arboviruses especially dengue and chikungunya in last few decades, making these viruses to gain a special attention. India is endemic for both dengue and chikungunya viruses, and presence of all the four serotypes of dengue virus have been reported from various parts of the country especially in Delhi, Kolkata, and Kerala.[1,2] Dengue and chikungunya viruses, though belong to two different family of *Flaviviridae* and *Togaviridae* respectively, share a common mosquito vector, that is, *Aedes agypti* in India.[1] *Aedes aegypti* abundantly found in tropical and subtropical countries is a principal vector for both of these viruses.[3] Every year the incidence of dengue and chikungunya increases abruptly between post-monsoon and early winter period as the climatic condition favors the mosquito breeding.[4] Both of these viral illnesses initially present with common signs and symptoms like fever, arthralgia, myalgia, etc., though joint pain is more severe and rashes are rarely seen in chikungunya fever.

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Dengue virus is a member of family *Flaviviridae* name originated from a Swahili word meaning "bone breaking fever," which is one of the chief complain of dengue fever (DF).[5] DF has wide range of presentations from self-limiting asymptomatic to severe form of disease in form of dengue hemorrhagic fever and dengue shock syndrome. Dengue virus is a positive sense single-stranded RNA virus having four serotypes referred as DENV1, DENV2, DENV3, and DENV4.[6] The presence of fifth variant DENV5 has not been documented yet in India.[7] All the four serotypes have been found to circulate in India specially in Delhi with predominance of one or more. Every year hospitals of Delhi are flooded with suspected case of dengue and majority of them are been diagnosed to have DF.

Chikungunya fever, name originated from the Makonde language in South Tanzania, meaning "to become contorted" refers to the posture of the patient due to severe joint pain.[8] Chikungunya virus, a member of Alphavirus of *Togaviridae* family, is a positive sense single-stranded RNA virus. Chikungunya fever became the topic of global concern after the outbreak in Indian Ocean Islands, and since then outbreaks have been reported from the different parts of the world.[9] Chikungunya virus shares the same mosquito vector *Aedes agypti* as dengue for its transmission. A single mutation in E1gp gene has greatly increased the viral survival in other mosquito species, that is, *Aedes albopictus* and thus expanded the spread of chikungunya virus beyond the tropical countries.[10] Recently, in 2016, Delhi witnessed a major outbreak of chikungunya fever with 9,793 laboratory confirmed cases.[11] A large number of patients were simultaneously diagnosed to have coinfection with dengue virus.[12]

This study was carried out to estimate the burden of dengue and chikungunya monoinfection and coinfection during 2016 Delhi outbreak.

Procedure

This study was conducted in the Department of Microbiology, Maulana Azad Medical College, New Delhi, during September 2016.

Venous blood samples were collected in a presterile plain vial from 200 suspected cases of dengue and/or chikungunya fever attending the Medicine Outdoor of associated Lok Nayak Hospital, New Delhi. Sera were separated and stored at −70° until further use.

All sera were tested for dengue fever using NS-1 Antigen ELISA kit (J. Mitra and Co. Pvt. Ltd., New Delhi) and IgM Antibody MAC ELISA kit (National Institute of Virology, Pune, India), and chikungunya fever using IgM Antibody MAC ELISA kit (National Institute of Virology, Pune, India) as per manufacturer's instructions.

OD values were recorded and results were interpreted as per the kit literature.

Results

This study included a total of 200 clinically suspected cases of dengue and/or chikungunya fever.

Dengue serology

Out of 200 clinically suspected cases, 34 (17%) were positive either for dengue NS-1 antigen or dengue IgM antibody or both. Among these, two were positive for NS-1 antigen alone, 22 were positive for IgM antibody alone, while 10 were positive for both. OD values for 32 samples fell in between the positive and negative cutoff and thus were reported equivocal [Table 1].

Chikungunya serology

Out of 200 tested cases, 77 (38.5%) were reactive for chikungunya IgM antibody. Out of these 77 positive cases, 41 (53.3%) cases were either positive or equivocal for dengue serology. Among them, 23 (29.9%) were positive for dengue serology [Table 2].

Sexwise distribution of dengue, chikungunya and coinfected cases clearly demonstrated male predominance with 61.8%, 63.6%, and 73.9%, respectively [Figure 1], while majority of the cases were from working age group of 21-40 years in all these three scenarios [Figure 2].

Table 1: Dengue serology

Test Result	NS1 Positive	NS1 Negative	NS1 Equivocal	Total
IgM Positive	10	22	0	32
IgM Negative	01	135	0	136
IgM Equivocal	01	31	0	32
Total	12	188	0	200

Table 2: Chikungunya and Dengue serology

Test Result	DEN Positive	DEN Equivocal	DEN Negative	Total
CHIK Positive	23	18	36	77
CHIK Negative	10	11	81	102
CHIK Equivocal	01	02	08	11
Total	34	31	135	200

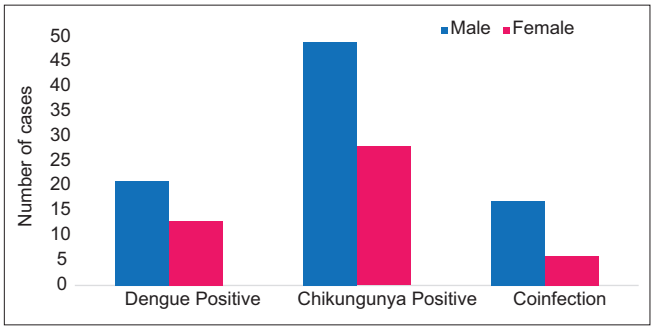

Figure 1: Sexwise distribution of cases

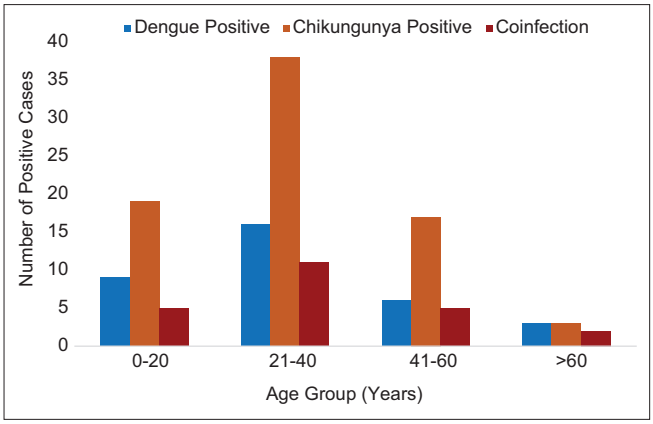

Figure 2: Age-wise distribution of cases

Discussion

India being an endemic country for arboviral fever like dengue and chikungunya, every or alternative year there is an outbreak of one or both. Chikungunya fever had been on declining trend since 2010, until the outbreak of 2016.[13] Delhi is among worst affected state when we talk of Northern part of the country. Many contributing factors are responsible for this tremendous rise in cases every year. Increasing population, immune status, rainfall, poor drainage system, artificial collection of water like pots, coolers, unused tyres, etc., contributing to increased mosquito population, ongoing construction work are the few important contributing factors. Circulation of both the viruses along with presence of common mosquito vector, coinfection with dengue and chikungunya is not uncommon. Both of these illnesses are associated with poor quality of life and dual infection further worsens the condition. It is a challenge for the treating physician while providing primary care to such patients. As per CDC (Centre for disease control and prevention), while providing primary treatment to a patient of Chikungunya, before starting NSAIDs (Nonsteroidal anti-inflammatory drugs) for fever and arthralgia, dengue infection should be ruled out, as these drugs can further complicate dengue illness because of their associated antiplatelet effect leading dengue hemorrhagic fever and even dengue shock syndrome. Similarly, only proving care for DF alone in a case of dual infection can lead to long-term arthralgia and other joint complications of chikungunya. Thus, it becomes important to diagnose the type of viral illness with which the patient is affected to guide the treating physician for appropriate primary care. Several studies from the northern part of India have highlighted chikungunya and dengue dual infections.

Several serological and molecular based diagnostic tools are available for diagnosis of dengue and chikungunya fever with varying sensitivity and specificity. ELISA-based test kits are mostly widely accepted and easily performed tests are most promising one. In our study, the incidence of dengue and chikungunya were found to be 17% and 38.5%, respectively, during the month of September 2016. Our findings for chikungunya incidence were similar with the study from Punjab during the same year.[14] Like all other studies, our findings too highlighted the male predominance, as they are more exposed to mosquito bite and majority of the cases were from the working age group of 21-40 years of age.[15]

Approximately 30% of the positive chikungunya cases in our study had coinfection with the dengue virus, which were a quite high number. This could be because of cross-reactivity of the ELISA kits. Cross-reactivity though rare but have been reported with other illness including dengue, although both these viruses belonged to two different families.[16] Suspicion of cross-reactivity was high as approximately 23.4% (18) of positive chikungunya cases had equivocal OD values for dengue serology, and if we combine both true positive dengue along with equivocal dengue numbers, more than half positive chikungunya cases (41, 53.3%) were associated with dengue. On the other hand, coinfection in our study was comparable with 23% and 25.3% from studies done by Mukherjee *et al.* and Kaur *et al.* during same year,.[12,15] even though the probability of cross-reactivity cannot be ignored until unless molecular techniques are being introduced to rule out cross-reactivity. Molecular techniques, being expensive, require expert personnel, making its availability difficult at all the testing centers.

Conclusion

India is a hyperendemic region for arboviruses like dengue and chikungunya which shares a common mosquito vector. Presence of both the vector and huge population at risk are the major contributors for the frequent outbreaks of these viral illnesses across the country, especially in Delhi. Post-monsoon and early winter favors the breeding of mosquito, along with simultaneous circulation of both the viruses had led to increase incidences of dual infection. Molecular techniques are more promising for diagnosing true coinfection as cross-reactivity though rare cannot be ignored. Thus, molecular methods if available should be employed to rule out cross-reactivity whenever the number of coinfections is abruptly higher than the normal expectancy. Both of these viral illnesses initially present with common signs and symptoms, it becomes difficult for the physician to make the diagnosis clinically. Chikungunya is associated with more severe and prolonged arthralgia leading to poor quality of life than dengue, although severe life threating complications are more common with dengue. So, it becomes a need to diagnose these viral illnesses simultaneously right in time for the proper primary care of the patients with or without coinfection to avoid fatal complications, as till date none of the antiviral agents have shown promising result against these viruses. Moreover, every dengue positive cases should be tested for chikungunya as well and vice-versa to diagnose coinfection.

Limitations

The major limitations of this study were, firstly, the small sample size. Secondly, our study did not employ any molecular techniques to identify possible cross-reactivity. Thirdly, it is a unicentric study and the results might not correspond to the real burden of the disease.

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Conflicts of interest

There are no conflicts of interest.

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