Concurrent Infections of Three Mosquito Borne Diseases-Dengue, Chikungunya and Malaria

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Abstract Kolkata, India is endemic for mosquito borne diseases like dengue, chikungunya and malaria. For monitoring, altogether 252 serum samples of fever cases were examined for dengue specific NS1 antigen and IgM and IgG antibodies and chikungunya specific IgM antibody. Their blood samples were also tested for malarial parasites. Out of 252 cases, 15 (5.95%), 16 (6.34%) and 18 (7.13%) were infected with dengue, chikungunya and malaria respectively. Amongst 15 dengue cases 10 (3.96%) were positive for both dengue IgM and IgG antibodies and 5 (1.98%) for NS1 antigen. Out of 18 malaria victims 14 (5.55%) and 4 (1.58%) were positive for Plasmodium vivax and Plasmodium falciparum respectively. During the present study, one case of concurrent infections of dengue and chikungunya and another case of concurrent infections of dengue, chikungunya and falciparum malaria were detected. Detail case report of the later has been described. This is the first ever report of concurrent infections of dengue, chikungunya and malaria.

Keywords Mosquito; Dengue; Chikungunya; Malaria; Plasmodium falciparum

Introduction Myers and Carey isolated dengue type 2 virus and chikungunya virus from a single blood sample taken from a patient in acute phase of a dengue-like disease in Vellore in South India in 1964 (Myers and Carey, 1967). In serial blood specimens collected from this patient the authors demonstrated an increase in antibody against both the viruses mentioned. Concurrent dengue virus and chikungunya virus infections during simultaneous outbreaks in Gabon in Africa in 2007 were documented, where either dengue-2 virus or chikungunya infections were identified in 321 patients at molecular level applying PCR technique, of which 8 had co-infections (Leroy et al., 2009). Concurrent isolation of dengue virus and chikungunya virus from a confirmed case imported from Singapore was described in Taiwan; the virus was detected through antibody neutralization and plaque purification techniques (Chang et al., 2010). Co-infection with dengue virus and chikungunya virus in 6 cases were obtained in Delhi, India through molecular techniques (Harendra et al., 2009). First report of mixed infection of dengue and malaria (P. falciparum) was documented by Charrel et al. in 2005 from France. Kolkata, India is endemic for mosquito borne diseases like dengue, chikungunya and malaria (both vivax and falciparum malaria) (Bandyopadhyay et al., 2009; Hati, 2004, 2006, 2011; Bhattacharya et al., 2009). Concurrent infections of dengue and malaria in Kolkata were recorded (Hati et al., 2012) through a longitudinal study, involving 6 years (2005-2010). During the present study from July 2011 to August 2012, altogether 252 serum samples of fever cases suspected to be suffering from viral diseases were tested for dengue specific NS1 antigen, and IgM and IgG antibodies and chikungunya specific IgM antibody by ELISA method. Peripheral blood of all those persons were also examined for detection of malaria parasites if any, through Giemsa’s stained thick and Leishman’s stained thin blood films under the oil immersion lens of the microscope using 10X eyepiece. Proper ethical permission was taken (No. VIHC/387).

Out of those 252 cases, 15 (5.95%), 16 (6.34%) and 18 (7.13%) were suffering from dengue, chikungunya and malaria respectively. Of those 15 dengue cases, 10 (3.96%) were reactive for both dengue IgM and IgG antibodies and 5 (1.98%) were reactive for NS1 antigen. Among 18 malaria cases, 14 (5.55%) had P. vivax trophozoites and gametocytes and 4 (1.58%) had P. falciparum rings (Table 1).
Table 1 Detection of dengue, chikungunya and malaria in 252 fever cases in Kolkata, India

| Total fever cases (%) | Total dengue cases (%) | Total Chikungunya cases (%) | Total malaria cases (%) | Case with concurrent infections of dengue & chikungunya (%) | Case with concurrent infections of dengue, chikungunya & P. falciparum malaria (%) |
|-----------------------|------------------------|----------------------------|-------------------------|----------------------------------------------------------|------------------------------------------------------------------------------|
| 252(100)              | 10(3.96)               | 5(1.98)                    | 16(6.34)                | 14(5.55)                                                | 4(1.58)                                                                       | 1(0.39)                                                                      |

Out of those infected persons, concurrent infections of dengue and chikungunya viruses were found in a boy of 11 years in November 2011. His serum was reactive for dengue specific IgM and IgG antibodies indicating secondary dengue where the IgM/IgG ratio was 0.91 which was lesser than 1.04 (WHO, 2009). His serum was also reactive for chikungunya specific IgM antibodies. Usual treatment responded and uneventful recovery of the patient was recorded.

Another male patient of Kolkata, aged 40 years reported with continuous fever (39°C) and chill and discontinuous rigor after 3 days of sufferings on 14 August, 2012. He had nausea, severe body and headaches, joint pains, hypotension and mild diarrhoea. He was advised for complete hemogram and peripheral blood for malarial parasites (MP). *Plasmodium falciparum* rings were detected (1% parasitaemia) on 15 August and his platelet count was 60,000/mm³. Other parameters of hemogram were normal. His treatment was started with Artesunate: Sulphadoxine: Pyrimethamine combination therapy along with Primaquine as per WHO regimen. Doxycycline 1g twice daily for 5 days was also administered along with Vitamin B complex. On 17 August the patient was free of rigor but remission of high fever (38.5°C with chill) did not occur. Symptoms like body and headaches, joint pains continued along with appearance of reddish skin rash (mixed type) on the face and extremities, appetite loss and weakness. He was admitted to a private nursing home and advised for re-examination of blood for MP and platelet count, blood culture for typhoid and serological tests for dengue specific NS1 antigen and IgM and IgG antibodies and chikungunya specific IgM antibodies by ELISA. His peripheral blood was negative for MP on 18 August. Test for typhoid was also negative but he was positive for NS1 antigen (cut off value 0.630, test value 1.876 & index value 2.98) and was also reactive for dengue specific IgM (cut off value 0.740, test value 2.877 & index value 3.89) and IgG (cut off value 1.23, test value 2.40 & index value 1.82) and chikungunya specific IgM (cut off value 0.740, test value 1.664 & index value 2.25) antibodies. This was a secondary dengue case i.e. he also suffered from dengue previously. Platelet count was 50,000/mm³. The patient was treated with paracetamol 1g twice a day for 5 days and sufficient amount of intravenous fluid was administered along with vitamin B complex. After 5 days the patient became afebrile and his platelet count rose near to normal.

Symptoms like continuous fever (39°C) and chill and discontinuous rigor, nausea, severe body and headaches, joint pains, hypotension and mild diarrhea, lower platelet count are indicative to joint infections. To separate out, discontinuous fever with chill and rigor along with nausea and mild diarrhoea are indications of malaria whereas, continuous fever with chill, severe body and headaches, joint pains, hypotension, lower platelet count are indications of dengue infection. Subsequent appearance of reddish skin rash (mixture of two types of skin rashes, one specific for dengue and another for chikungunya) on the face and extremities indicates joint viral infections, which was confirmed by ELISA. Loss of appetite and weakness were generalized symptoms for continuous sufferings.

The present study indicates intense activities of two mosquito borne viruses (dengue and chikungunya) and two malaria parasites (*P. vivax* and *P. falciparum*) in the city of Kolkata, India.

Though previous records of concurrent infections of dengue and chikungunya (Myers and Carey, 1967; Leroy et al., 2009; Chang et al., 2010; Harendra et al., 2009) and concurrent infections of dengue and malaria (Charrel, 2005; Hati et al., 2012) exist, this is the first ever report of concurrent infections of dengue, chikungunya and malaria (*P. falciparum*).
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