Investigation of an outbreak of chikungunya in Malegaon Municipal areas of Nasik district, Maharashtra (India) and its control

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Abstract

Background & objectives: An outbreak of chikungunya fever occurred in Malegaon town of Nasik district of Maharashtra state, India during February and March 2006. A total of 4530 fever cases were reported during this period including 1781 cases which were admitted in different hospitals of the town. An entomological and epidemiological investigation was carried out in the affected villages during the outbreak to study the possible causes of the outbreak and to isolate the virus responsible.

Methods: Entomological evaluation was done as per WHO guidelines. Sera samples were collected by venipuncture from clinically suspected chikungunya patients in hospitals and also during house-to-house survey in affected villages. IgM antibodies to dengue virus were detected using IgM capture ELISA (PANBIO) and by “Haemagglutination inhibition test” for detection of antibodies against Chikungunya virus. Acute sera samples were inoculated in cell lines for virus isolation. The isolates were confirmed by RT-PCR.

Results: On investigation, it was found that water storage containers like cement tanks, plastic containers or earthen pots placed in front of the individual houses were the potential breeding sites for Aedes aegypti. Entomological survey carried out in the most affected areas revealed high Aedes indices. House, container and breteau indices were found to be 27.2, 16.19 and 35.1, respectively. Out of the 13 acute sera samples collected, virus was isolated in 10 samples. The isolates were confirmed by RT-PCR and sequencing using primers from nsP1 gene of Chikungunya virus (CHIKV, Accession No. EF077609, EF077610). Of the 17 convalescent sera tested, significant level of HI antibodies to CHIKV was detected in five samples. One sample was positive for IgM antibodies against dengue virus. Based on clinico-epidemiological features and laboratory findings, the illness was confirmed to be of chikungunya viral disease.

Conclusion: Control measures targeting the vector population and personal protective measures against the mosquito bites were instituted. Extensive IEC campaign with the involvement of community and religious leaders helped in containment of the disease.

Key words Aedes aegypti – chikungunya – dengue – outbreak – vector control

Introduction

In February–March 2006, a perplexing febrile illness associated with crippling arthralgia, involving large number of people was observed in Malegaon taluka, Nasik district, Maharashtra. The illness was self-limiting, and the patients were cured by symptomatic therapy in 5–6 days. However, during acute stage,
the patients presented with high grade fever with chills associated with severe pain in joints to such an extent that the patient could not straighten up and walk and hence adopted a flexed posture. Although incapacitating, deaths were not reported due to the disease. Dengue and malaria, which have been reported in the past from Malegaon, formed the initial differential diagnosis. However, since Chikungunya virus (CHIKV) outbreak had been reported from the adjoining states of Andhra Pradesh during the same period, the possibility of CHIKV infection could not be ruled out on clinical profile. About 1543 blood slides were collected from fever cases between 27 February and 15 March 2006 by local health authorities. All blood smears were found to be negative for malaria.

A multi-disciplinary team visited Malegaon town from 23 to 27 March 2006 to conduct epidemiological, microbiological and entomological investigations to confirm the etiology of the outbreak. This study describes the findings of the outbreak investigations at Malegaon.

Material & Methods

Study area: District Nasik lies between 19°35’ and 20°52’ north latitude; and 73°16’ and 74°56’ east longitude. It is a famous holy tirtha and headquarter of the division. Malegaon City with a population of approx. 4,60,000 is located 280 km northeast of Mumbai. It is a densely populated city averaging about 27,000 people/km². Two major rivers namely Girna and Mosam pass through the town. The average rainfall in the area is about 280 cm but is highly seasonal due to monsoon. It is mainly drought during summer months leading to acute shortage of water in the area. In some of the areas the municipal water supply is only for half an hour duration with extremely low water pressure. Most people are employed in power loom industry. Two municipal hospitals—Ali Akbar Hospital and S.N. Wadia Hospital; and one State Government Rural Hospital cater to the health needs of the people.

A multi-disciplinary team visited Malegaon and carried out epidemiological and entomological investigations collecting samples for laboratory confirmation. Epidemiological investigations were carried out by discussion with the district health authorities, examining patients in the hospitals outpatient departments (OPDs) and wards, discussing with treating physicians, analyzing records and reports of all the patients in the three hospitals and house-to-house visits of representative affected areas.

Entomological survey for the mosquito vector was undertaken as per the standard WHO guidelines in the most affected localities. Door-to-door search was done using single larval technique to find the larval breeding in all the wet containers present in and around the houses. A total of 106 adult mosquitoes were collected from indoor houses and nearby containers having Aedes breeding in the affected localities—Madinabad, Auyasha Nagar, Ramjanpura, Camp area and Kamalpura during the survey for identification and brought to NICD for virus isolation. Adult and larval Aedes were identified using standard identification keys.

Sample collection: In all 13 acute sera samples of the clinically suspected cases of chikungunya fever were collected through veinipucture from the patients with symptoms of fever with arthralgia of one to five days duration from hospital OPD. A total of 17 blood samples were collected during the house-to-house survey from convalescing patients who had recovered from clinical illness 10 days to two months period before collection of samples. The samples were transported under cold conditions to NICD, Delhi for the analysis.

The samples from acute cases were inoculated in mouse neuroblastoma (MNA) cell lines. The mosquitoes were pooled, squashed and inoculated in batches in MNA cells. After incubation for 5–7 days, the virus was detected by haemagglutination test and confirmed by RT-PCR using nsP1 primers of
CHIKV\textsuperscript{4}. The representative strain was sequenced employing big dye terminator cycle sequencing ready reaction kit (Perkin-Elmer, Applied Biosystems, USA) on an ABI 310 sequencer and was subjected to Blast analysis.

The convalescing samples were tested for IgM antibodies against dengue virus using IgM Capture ELISA (PANBIO) and by “Haemagglutination inhibition test”\textsuperscript{3} for detection of antibodies against CHIKV. The chikungunya antigen was obtained from the National Institute of Virology, Pune.

Results

Clinico-epidemiological features: The records revealed that sporadic fever cases were being reported regularly, however, there was sudden increase in number of cases from 27 February 2006 onwards. A total of 4530 fever cases were reported from 27 February to 25 March 2006 from Malegaon. Of these, 3412 were reported from Ali Akbar Hospital, Wadia Hospital and Rural Hospital; and 1118 cases were detected by the health workers during active surveillance. A total of 1781 cases were admitted during this period in these three hospitals for fever with arthralgia. About 1398 (78.5\%) patients were discharged on the same day and 358 (20.1\%) were admitted for one day. The remainder (25; 1.4\%) were admitted for 2–4 days. Maximum number of cases 507 and 485 were reported on 17 and 18 March respectively, thereafter cases and hospital admissions declined (Fig. 1). Fever cases were reported from urban and peri-urban areas in all the wards, however, the most affected localities were Auyasha Nagar, Ramjanpura, Kamalpura, Nayapura and Islam Nagar. The onset of illness was observed to be acute with moderate to high fever, chills and associated joint pains. The joints affected were knee, ankle, wrist, elbow and small joints of the hands. In most of the cases, the patients found it difficult to stand up because of joint pain. No haemorrhagic manifestation was observed or re-

![Fig. 1: Date-wise fever patients and indoor patients recorded in Malegaon City, Nasik](image-url)
reported. Lymphadenopathy and rash were encountered rarely. No deaths were reported due to this disease. Both the genders (Male : Female—52.2 : 46.7%) were affected however majority of cases were observed in persons between 11 and 50 years of age (Table 1). All the patients were given symptomatic treatment with paracetamol/NSAIDS (Non-steroidal anti-inflammatory drugs) and were advised rest.

For field epidemiological studies, house-to-house visits were conducted in Auyasha Nagar, Ramjanpura and Kamalpura. These areas are dominated by illiterate labourers with very low socioeconomic status working in power loom industry, most of them belonging to muslim community. Each house had 5–10 people living in small single room. The localities were congested with poor sanitary conditions. Approximately, 120 houses were visited. Field visits indicated that the illness was self-limiting and the fever lasted for 2–7 days. However, few patients complained of joint pain even after a month. Two to three people were affected in the same house, symptoms of illness appeared within 2–3 days.

**Entomological survey:** Entomological survey was carried out in seven localities for the prevalence of the vector. It was observed that because of low water pressure people had designed special type of hand pumps to draw water from municipal line. Each house had one or two big overhead and underground cement tanks, along with multiple types of small containers for storage of water. The containers were left uncovered or partially covered in most of the places. During the survey, 490 houses were examined for mosquito breeding. Of these 136 (28.1%) were found to positive for *Aedes* breeding (house index 27.2%). Of 1062 containers examined *Aedes* breeding was observed in 172 containers (container index 16%). The breteau index was 35.1 (Table 2). Of the seven localities surveyed, *Aedes* breeding was detected in all, and five localities had house index more than the critical index of 10%. The main containers positive for *Aedes* breeding were cement tanks (19.91%), plastic and tin containers (17.55%), and earthen pots (7%) (Table 3).

**Laboratory results:** Of the 13 serum samples inocu-

<table>
<thead>
<tr>
<th>Age group</th>
<th>Rural SN Wadia Hospital</th>
<th>Ali Akbar Hospital</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤1</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>4 (0.2)</td>
<td>3</td>
</tr>
<tr>
<td>&gt; 1–11</td>
<td>51</td>
<td>111</td>
<td>23</td>
<td>185 (10.4)</td>
<td>111</td>
</tr>
<tr>
<td>&gt; 11–21</td>
<td>131</td>
<td>202</td>
<td>112</td>
<td>445 (25)</td>
<td>269</td>
</tr>
<tr>
<td>&gt; 21–31</td>
<td>97</td>
<td>201</td>
<td>132</td>
<td>430 (24.1)</td>
<td>223</td>
</tr>
<tr>
<td>&gt; 31–41</td>
<td>94</td>
<td>161</td>
<td>117</td>
<td>372 (20.9)</td>
<td>168</td>
</tr>
<tr>
<td>&gt; 41–51</td>
<td>55</td>
<td>75</td>
<td>69</td>
<td>199 (11.2)</td>
<td>103</td>
</tr>
<tr>
<td>&gt; 51–61</td>
<td>25</td>
<td>30</td>
<td>24</td>
<td>79 (4.4)</td>
<td>38</td>
</tr>
<tr>
<td>&gt; 61–71</td>
<td>16</td>
<td>40</td>
<td>11</td>
<td>46 (2.6)</td>
<td>21</td>
</tr>
<tr>
<td>&gt; 71–81</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>16 (0.9)</td>
<td>11</td>
</tr>
<tr>
<td>&gt; 81–91</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1 (0.1)</td>
<td>1</td>
</tr>
<tr>
<td>&gt; 91–100</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>4 (0.2)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>469 (26.33)</td>
<td>823 (45.95)</td>
<td>489 (27.46)</td>
<td>1781 (100)</td>
<td>94 (53.23)</td>
</tr>
</tbody>
</table>

Figures in parentheses represent percentages.
lated in MNA cell lines, CHIKV was isolated in 10 samples. The isolates were confirmed by RT-PCR using primers from nsP1 gene of CHIKV. Two representative strains were sequenced and confirmed by blast analysis (Accession No. EF077609, EF077610). No virus was isolated from pools of adult mosquitoes. Of the 17 convalescent sera tested, five samples had HI titres of 1:40, one each had titre of 1:80 and >1:160 suggesting chikungunya infection. Dengue IgM antibodies were detected in one serum sample.

### Discussion

Chikungunya is a viral fever caused by an alpha virus belonging to family of Togaviridae viruses that is spread by the bite of *Aedes aegypti* mosquito. CHIK activity in Asia was first documented since its isolation in Bangkok, Thailand in 1958. Virus continued to be transmitted till 1964. It re-emerged again in mid-1970s and disappeared by late 1970s. Chikungunya infection is not new to India. Prior to the present epidemic, outbreaks of chikungunya have...
been reported from Calcutta in 1963, southern India (Vellore and Madras) in 1964, and 10 years later in 1973 from Sholapur district, Maharashtra.

It was estimated that the outbreaks in Calcutta and south India, involved large number of people having characteristic joint pain affecting both genders equally accompanied by febrile illness. In the present outbreak also classical symptoms of fever with joint pain were observed. In the acute stage patient could not walk and adopted a bent/flexed posture due to pain. Present outbreak also involved large number of people as has been observed in previous outbreaks. About 39% patients sought admission in the hospital probably because of high-grade fever and crippling arthralgia. Of these, 1756 (98.6%) were discharged with in a day after reassurance and supportive treatment.

*Ae. aegypti* was observed in the local houses with high *Aedes* larval indices above the critical limit as per the WHO criteria. The occurrence of multiple disease cases (two to three individuals) in the same family in short period of time indicates mechanical transmission of the disease. Cement tanks were found to be the most preferred containers for *Aedes* breeding because water in these containers was never emptied and was replenished periodically, making them the perennial breeding sites. The other preferred breeding sources were plastic and tin containers. Besides these, other containers positive for *Aedes* breeding were earthen pots as they were left open or had defective lid.

In the present outbreak, clinico-epidemiologically it was difficult to differentiate dengue infection from CHIKV infection. Both the diseases are transmitted by the same vector and have similar clinical presentation. The diagnosis was established by serology, virus isolation and identification by sequencing the nsP1 genome. In this episode the attempt was made to isolate the virus in murine neuroblastoma (MNA) cells as literature suggested that the virus might be neurotropic in nature. Cases of meningoencephalitis caused by this virus were reported from Re-union Island. The mosquito cell lines which were used in previous outbreaks were not immediately available for use. CHIKV grew luxuriantly in MNA cells which was confirmed by RT-PCR and sequencing.

Indoor fogging with pyrethrum extract was suggested around 50 houses of fever cases as per the directions of National Vector Borne Disease Control Programme (NVBDCP) to cut down the transmission of the disease. Larvicide (Temephos) was used in water containers holding water for bathing and washing purposes for controlling the vector population. Personal protective measures like use of mosquito repellents and mosquito nets were also suggested.

The outbreak was contained; however, some questions remained unanswered. Why did the outbreak re-occur after lapse of almost three decades remain unclear? However, some serological studies conducted in Calcutta and Madras City suggest that waning herd immunity could be one of the reasons for large epidemics occurring at intervals of several years as susceptible population accumulates. Calcutta experienced the CHIK outbreak in 1963, serosurvey conducted in 1994 showed seropositivity of 4.37%, highest (12.5%) being observed in age group of 51 to 55 years. Similarly, retrospective serosurvey conducted in samples collected in 1956 from Madras City showed seropositivity of 10.8%, majority in age group of above 40 years.

Since 2004, a large outbreak of chikungunya infection was ongoing in Indian Ocean, affecting the popu-
lations of Comoros, Mayote, Madagascar, Mauritius, Seychelles and Re-union. In Re-union approximately one-third of the total population were reported to be infected by April 2006. Analysis of the strains from this outbreak indicated a specific change at position 226 of E1 protein. This mutation helped the CHIKV to multiply without the need of cholesterol, which viruses normally need to infect the cells of the host. Because mosquitoes often do not have enough cholesterol for viruses to efficiently affect their cells, it has been hypothesized that after mutation in E1 protein CHIKV could survive and multiply better in mosquitoes, which could have contributed to its rapid spread involving large number of people. The Indian isolates need further characterization and study of E1 protein region. There is also a need to maintain serosurveillance to understand the epidemiology of the disease, occurrence of sub-clinical infection and immunity. Until then the outbreaks need to be prevented by vector source reduction and ensuring adequate and proper water supply system.

References


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