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Dengue serosurveillance in Kolkata, facing an epidemic in West Bengal, India

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Abstract

Background & objectives: A dengue outbreak occurred throughout West Bengal, India starting from August 2005. The objectives of this study were: (i) documentation of suspected cases, confirmed by MAC ELISA; (ii) a longitudinal serosurveillance of dengue in Kolkata by detecting dengue-specific IgG and IgM antibodies in suspected cases and to note the seasonal incidence; (iii) to get information about proportion of primary, secondary and old dengue cases and persons free from dengue in the population tested; and (iv) to get some idea to forecast advent of dengue and dengue epidemic/precipitation of DHF.

Methods: The epidemiological data were collected from different sources including Calcutta School of Tropical Medicine and Government of West Bengal. For serosurveillance in Kolkata, serum of each suspected patient was examined for detection of IgG and IgM antibodies using the microwell ELISA dengue fever kit.

Results: Altogether, 6293 ELISA IgM reactive cases were documented out of 12,059 persons (52.18%) tested in the whole of West Bengal, involving 18 districts with 34 deaths. Serosurveillance of 1668 persons from August 2005 to December 2007 in Kolkata showed that only 18.1% (302) suffered from dengue, 6.03% (101) from primary, 12.03% (201) from secondary dengue, 20.44% (341) were not sufferers of dengue and 61.45% (1025) were old dengue cases. Though stray dengue cases were found perennially, most of the cases occurred in the post-monsoon season, with a peak in September. Of dengue patients, 63.24% (191) were males. Secondary dengue cases outnumbered primary dengue cases. So, possibility of DHF would remain in all age groups and in those persons who suffered from dengue on earlier occasion. Dengue cases drastically reduced in two subsequent post-epidemic years (2006 and 2007).

Interpretation & conclusion: Dengue serosurveillance studies may give some idea about advent, intensity, transmission season, seasonal incidence, waxing and warning, and impending epidemic of dengue and DHF. A large-scale active longitudinal serosurvey along with the study of vector capacity and vector competence would provide more correct information.

Key words Dengue – DHF – IVD microwell ELISA – Kolkata – serosurveillance

Introduction

Dengue broke out in epidemic form during August to November 2005, affecting 18 districts of West Bengal, India, including the city of Kolkata, claiming 34 lives. Altogether, 12,059 were tested during that period and 6293 (52.18%) were ELISA IgM reactive. Kolkata was worst affected and it alone shared 63.76% of those dengue cases (4013), with 13 deaths[1]. Such cases began to reduce drastically from December 2005. Keeping this in background, a longitudinal serosurvey of dengue through detection of specific IgG and IgM antibodies in the sera of fever cases, suspected to be suffering from dengue was planned just from the beginning of the outbreak of the episode (August 2005) which was continued up to

December 2007. These facilities for surveillance of dengue involving ELISA technique were not available beforehand, which might provide a lot of information related to some epidemiological aspects of dengue and dengue haemorrhagic fever (DHF).

The aims and objectives of this study were: (i) documentation of suspected cases confirmed by MAC ELISA and brief statement of epidemic outbreak of dengue faced by West Bengal in 2005; (ii) a longitudinal serosurveillance of dengue in Kolkata by detecting dengue-specific IgG and IgM antibodies in the sera of suspected fever cases through August 2005 to December 2007 and to note the seasonal prevalence; (iii) to get information about existence and proportion of primary, secondary and old dengue cases and cases free from dengue in the population tested; and (iv) to get some clue to forecast advent of dengue and dengue epidemic/precipitation of DHF. This paper consisting of two parts, i.e. documentation of the outbreak and serosurveillance in Kolkata that deals with findings which were generated during the study.

Material & Methods

The epidemiological data were collected from different sources including Calcutta School of Tropical Medicine and Government of West Bengal. For dengue longitudinal surveillance in Kolkata, the blood was collected from each patient suspected to be suffering from dengue in each month starting from August 2005 to December 2007, for detection of both dengue-specific IgG and IgM antibodies in the blood of each patient and sent to the Goutam Laboratories situated in central Kolkata. In every case the blood was collected at least five days after the onset of fever, allowing IgM antibodies to appear, if present. Age and sex of each patient were noted.

Specific dengue IgG and IgM antibodies if present, were detected using the IVD microwell ELISA dengue fever kit[2], from each sample of processed serum, following the directions of manufacturing company. The result was obtained by the patient on the same day of blood test.

The test procedure: To 200 µl sample diluent, 10 µl serum was added. About 100 µl dilute sample was placed in the well, which was kept at room temperature for 20 min. This was discarded and the well was washed with wash buffer 20x (prepared with 5 ml of distilled water and 250 µl of wash buffer) for three times, at the interval of 15 sec. Then to the well 100 µl of enzyme conjugate was added, kept for 20 min and washed thrice. After that 100 µl of TMB substrate was added to the well, which was kept for 10 min in room temperature in a dark place. Thereafter 100 µl of stop solution was added and the sample was read in ELISA reader at 450/630 nm. The procedure was same for IgG and IgM antibodies, using appropriate kit. Cut-off point was determined (<0.9 = non-reactive; 0.9–1.1 = borderline; and >1.1= reactive).

Interpretation of the test: In the sera of some patients no IgG or IgM antibodies were present. Hence, they were not suffering from dengue. The serum of those patients that contained only IgG antibodies did suffer from dengue, sometime ago, but not during the present study. They were regarded as old cases having previous antibodies (IgG) in their blood. The serum of those patients that contained only IgM antibodies were regarded as primary cases of dengue, i.e. they were attacked with dengue for the first time. They did not suffer from dengue beforehand. The serum of those patients that contained both IgG and IgM antibodies were secondary dengue cases. They did suffer earlier from dengue for that reason IgG antibodies were there in addition to IgM antibodies which appeared as a result of fresh infection from another type of dengue virus, not similar to previous one.

Results

A comprehensive picture of dengue epidemic occurred in West Bengal state is given in Table 1. For surveillance of dengue in Kolkata, altogether 1668 serum samples were examined (868 in 2005, 627 in 2006 and 173 in 2007). A total of 302 dengue (both primary and secondary) cases were obtained, constituting 18.10% of the people examined. Year-wise distribution of cases were 224, 62 and 16 indicating 25.8,

Table 1. District and area-wise distribution of confirmed cases and deaths due to dengue/DHF in West Bengal

District/Area	Number	Cases (%)	Deaths
Kolkata	4013	63.76	13
24 Parganas (N)	524	8.33	7
Howrah	264	4.19	4
24 Parganas (S)	253	4.02	3
Hooghly	171	2.71	0
Maldah	155	2.46	0
Salt Lake City	135	2.15	1
Burdwan	104	1.65	1
Nadia	85	1.35	1
Midnapur (W)	50	0.75	0
Birbhoom	47	0.7	1
Midnapur (E)	27	0.43	0
Dinajpur (S)	13	0.21	0
Bankura	9	0.14	1
Darjeeling	4	0.06	1
Purulia	2	0.03	1
Dinajpur (N)	2	0.03	0
Coochbehar	2	0.03	0
Jalpaiguri	1	0.02	0
Outside State	16	0.24	0
Not known	416	6.61	0
Total	6293	100	34

9.88 and 9.24% of fever cases suffering from dengue in 2005, 2006 and 2007, respectively.

Total number of primary dengue cases was 101 (33.44%) of all dengue cases. Number of primary dengue cases was 77 (34.37%) in 2005, 23 (37.09%) in 2006 and 1 (6.25%) in 2007; whereas of 201 total secondary dengue cases, 147 (65.62%), 39 (62.90%) and 15 (93.75%) were found in 2005, 2006 and 2007 respectively. As a whole secondary dengue cases constituted 66.55% of all dengue cases.

This surveillance study indicated that as a whole 20.44% (341) of fever cases examined were not suffering from dengue, the range varied from 18.81% (118) in 2006 to 21.54% (187) in 2005. It also pointed out that 61.45% (1025) of the surveyed population were old dengue cases. Such old dengue cases amounted to 457 (52.65%) in 2005, 447 (71.29%) in 2006, and 121 (69.94%) in 2007 (Table 2).

The age gradation of primary and secondary dengue cases is presented in Table 3. It was found that 79.2% of primary dengue cases occurred in age group 0–20 yr. Only 1.98% of cases were found in the age group 41–60 yr. No primary dengue case was found in persons beyond 50 yr of age.

Table 2. Serological studies on dengue in Kolkata (2005–07)

Year	Total No. of sera of fever cases tested	No. of patients having no dengue antibody (not suffering from dengue)	No. of patients having IgG antibody only (Old dengue cases)	No. of patients having either IgM or both IgM and IgG antibodies (Total dengue cases)	No. of patients having IgM antibody alone (Primary dengue cases)	No. of patients having both IgM and IgG antibodies (Secondary dengue cases)
2005 (Aug–Dec)	868	187 (21.54)	457 (52.65)	224 (25.8)	77 (8.87)	147 (16.93)
2006 (Jan–Dec)	627	118 (18.81)	447 (71.29)	62 (9.88)	23 (3.66)	39 (6.22)
2007 (Jan–Dec)	173	36 (20.8)	121 (69.94)	16 (9.24)	1 (0.57)	15 (8.67)
Total	1668	341 (20.44)	1025 (61.45)	302 (18.1)	101 (6.05)	201 (12.06)

Figures in parentheses are percentage.

Table 3. Age gradation of 302 patients suffering from primary and secondary dengue infection

Age (yr)	Patients suffering from primary dengue	Patients suffering from secondary dengue	Total cases
0–5	27 (26.73)	15 (7.46)	42
6–10	21 (20.79)	17 (8.45)	38
11–20	32 (31.68)	59 (29.35)	91
21–30	12 (11.88)	49 (24.37)	61
31–40	7 (6.93)	25 (12.43)	32
41–50	1 (0.99)	15 (7.46)	16
51–60	1 (0.99)	10 (4.97)	11
61–70	0	5 (2.48)	5
71–80	0	3 (1.49)	3
>80	0	3 (1.49)	3
Total	101 (100)	201 (100)	302

Figures in parentheses are percentage.

Secondary dengue cases were distributed in all age groups (45.26% in 0–20 yr age group and 10.43% in >50 yr of age) (Table 3). Only 3.12% of the patients (32) that had suffered from dengue earlier were in the age groups 1–10 yr. Such old dengue cases were found more in the age groups 11–50 yr (769, i.e. 77%), than in any other age group. Test samples of 81.8% of patients (279) where no dengue antibody was found were in 0–30 yr age groups and about 22.8% (78) in the age group 0–5 yr, 10.8% (37) in the age group 6–10 yr, and 26.9% (92) in the age group 11–20 yr.

Month-wise number of total primary and secondary dengue cases is given in Tables 4 and 5. Number of primary dengue cases was highest in September (41) in 2005, in October (11) in 2006, and in 2007, only one primary case (out of 16 cases) was found in November. Number of secondary dengue cases was also highest in September (69) in 2005, in October (20) in 2006, and in December (5) in 2007 (Tables 4 and 5).

Number of total cases of dengue was highest in September (117), followed by October (105) and November (45). Of all the patients (302) suffering

Table 4. Serological studies on dengue in Kolkata (2005–07): monthwise analysis of cases

Month	2005						2006						2007					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Jan							6	2	3	1	–	–	4	2	2	–	–	
Feb							1	1	–	–	–	–	6	3	2	–	1	
Mar							1	1	–	–	–	–	3	1	2	–	–	
Apr							3	–	1	1	1	50	1	–	1	–	–	
May							4	1	2	–	1	100	2	1	1	–	–	
Jun							7	1	5	–	1	100	5	1	4	–	–	
Jul							39	8	26	1	4	80	3	–	3	–	–	
Aug	43	16	15	8	4	33.3	51	10	38	2	1	33.3	19	1	17	–	1	
Sep	506	117	279	41	69	62.7	40	7	28	–	5	100	15	5	8	–	2	
Oct	212	29	111	19	53	73.6	258	44	183	11	20	64.51	39	10	27	–	2	
Nov	79	16	34	9	20	69.0	185	40	134	7	4	36.36	44	9	30	1	4	
Dec	28	9	18	–	1	–	32	3	27	–	2	100	32	3	24	–	5	
Total	868	187	457	77	147	65.62	627	118	447	23	39	62.90	173	36	121	1	15	93.75
				224*						62*						16*		

1–Total sera tested; 2–Cases that were not suffering from dengue; 3–Old dengue cases; 4–Primary dengue cases; 5–Secondary dengue cases; 6– Percentage of secondary dengue cases; *Total number of primary and secondary dengue cases.

Table 5. Month-wise distribution of primary, secondary and total dengue cases during dengue surveillance in Kolkata

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Primary dengue cases													
2005	–	–	–	–	–	–	–	8	41	19	9	–	77
2006	1	0	0	1	0	0	1	2	0	11	7	–	23
2007	–	–	–	–	–	–	–	–	–	–	1	–	1
Total	1	–	–	1	–	–	1	10	41	30	17	–	101
Secondary dengue cases													
2005	–	–	–	–	–	–	–	4	69	53	20	1	147
2006	0	0	0	1	1	1	4	1	5	20	4	2	39
2007	0	1	0	0	0	0	0	1	2	2	4	5	15
Total	0	1	0	1	1	1	4	6	76	75	28	8	201
Total dengue cases													
2005	–	–	–	–	–	–	–	12	110	72	29	1	224
2006	1	–	–	2	1	1	5	3	5	31	11	2	62
2007	–	1	–	–	–	–	–	1	2	2	5	5	16
Total	1	1	–	2	1	1	5	16	117	105	45	8	302

from dengue, 191 (63.24%) were males. Age and sex-wise distribution of dengue cases is given in Table 6.

Table 6. Surveillance of dengue cases: age and sex-wise distribution

Age group (yr)	Male	Female	Total
0–5	22	20	42
1–10	24	14	38
11–15	40	16	56
16–20	24	11	35
21–25	21	10	31
26–30	18	12	30
31–35	5	4	9
36–40	17	6	23
41–45	3	2	5
46–50	6	5	11
51–55	3	4	7
56–60	2	2	4
>60	6	5	11
Total	191	111	302

Statistical evaluations: The distribution of dengue was observed to be maximum in the age groups 11–20 yr and gradually declined with increase of age. At young age (0–10 yr), primary dengue infection was more frequent than secondary infection. As age increased (from age group 10–20 yr), secondary dengue cases occurred more frequently as compared to primary dengue infection. In >41 yr age groups, secondary dengue cases dominated primary dengue cases significantly. When compared, secondary dengue cases were always higher than primary dengue cases. There was significant decline in the frequency of cases tested positive for dengue from 2005 through 2007. The rate of decrease in total dengue cases in the period 2006–07 was higher (3.875 fold) than that in 2005–06 (3.615 fold). The decline in 2006–07 was steeper than that in 2005–06. Both primary dengue cases and secondary dengue cases declined steeply in 2005–06 than in 2006–07. Fig. 1 exhibits the seasonal pattern observed with the data in September, October and November. Number of cases decreased over the years, but the pattern approximately remained the same. Figs. 2 and 3 showed the actual

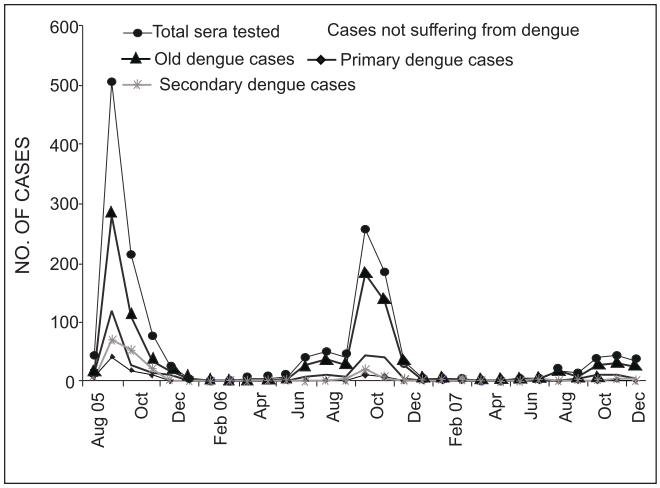

Fig. 1: Serological studies on dengue in Kolkata (Aug 2005– Dec 2007): month-wise analysis

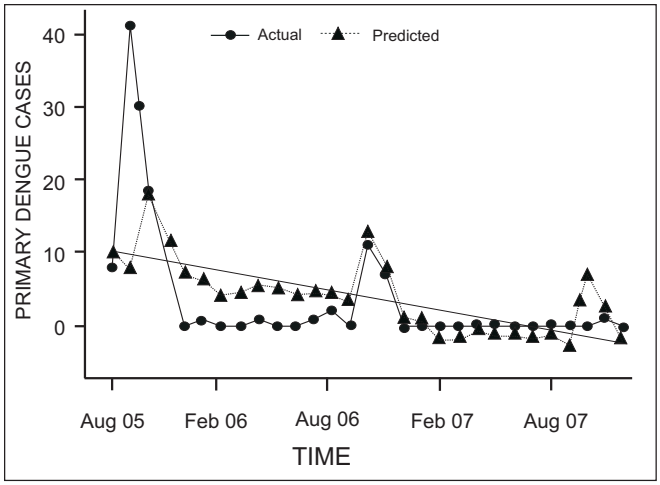

Fig. 2: Actual and predicted figures for primary dengue cases showing the trend line

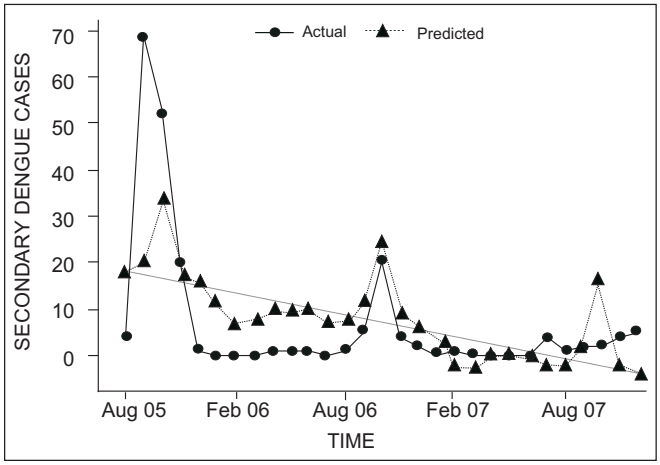

Fig. 3: Actual and predicted figures for secondary dengue cases showing the trend line

and predicted values for the primary and secondary dengue cases respectively. There is a trend line indicating the long-term movement of the data.

Discussion

The nature and extent of outbreak of dengue in 2005 in West Bengal was deeply investigated and a maiden attempt was made to confirm dengue cases employing MAC ELISA in this study. This extensive outbreak deserves serious attention for more than one reasons. Firstly, dengue not only affected the City of Kolkata, but also spread to 18 districts of the State of West Bengal, which was spectacular and unprecedented. Dengue was essentially an urban disease, confined to Kolkata in this state. But in 1994 epidemic of dengue was recorded in rural areas, with no death. Dengue virus was isolated from locally caught *Aedes aegypti*[3]. Some reports of sporadic outbreak of dengue in rural areas are available[4–8]. But this present outbreak took the form of a widespread epidemic affecting almost the entire state. Secondly, several deaths occurred due to DHF which was a new threat in the rural West Bengal. Thirdly, though dengue was first documented in Kolkata (Calcutta) in 1824[9] and several epidemics took place in the city during the years 1836, 1906, 1911 and 1972 (affecting 40% of the city people)[9], no large-scale laboratory confirmation of dengue cases and accurate measure of the number of cases of dengue was attempted. Using modern diagnostic tools detecting IgM antibodies it was possible to do so and 52.18% of 12,059 people tested was ELISA IgM reactive. This was a maiden serological study using MAC ELISA technique.

Facing this epidemic and keeping in view that Kolkata was worst-affected, dengue surveillance in Kolkata was conducted from August 2005 to December 2007, on the basis of both dengue specific IgG and IgM antibodies that would be able to provide several basic information, hitherto not explored.

On the basis of the data collected, the population of this study could be categorised in several district groups : (i) only 18.10% (302) of them suffered from

dengue; (ii) 6.05% (101) from primary dengue; (iii) 12.05% (201) from secondary dengue; (iv) 20.44% of the population (341) were not sufferers of dengue, having no dengue antibodies in their blood; (v) 61.45% (1025) of the people were also not suffering from dengue at the time of study, but they were old cases of dengue who had suffered sometime in the past. No such information was available till now. This observation to some extent reflected the stratification of the people of the community in Kolkata. This was a sort of small-scale passive study. A large-scale active serosurveillance would be necessary to get the clear picture of these important epidemiological determinants.

This study clearly depicted the seasonal incidence of dengue in Kolkata. During the post-monsoon season majority of the cases were reported, with a peak in September where majority of cases are reported. Distribution of stray cases throughout the year pointed out that perennial transmission is going on in Kolkata which requires attention, because this can be effectively reduced through proper vector control measures.

The number of secondary dengue cases was much more than that of primary dengue cases (201 : 101). Year-wise ratio was 147 : 77 (2005), 39 : 23 (2006) and 15 : 1 (2007). So possibility of precipitating DHF/DDS would remain. As secondary dengue cases were distributed in all the age groups, the chance of developing DHF/DDS could be there in any age group.

Again, according to this study a large proportion of people in Kolkata harbour IgG antibodies (61.4%) which seems to be natural, because dengue is endemic in this city. They are also potentially vulnerable as they can be attacked with a different type of dengue virus and in such cases immunological disturbances precipitating to DHF/DDS may occur. This seems to be a significant finding.

Out of the three study years, 2005 was worst-affected with large number of cases (224). But there was sizeable reduction of dengue cases in two subsequent years, i.e. in 2006 (9.88%) and 2007 (9.22%) in comparison to 2005 (25.80%), when epidemic occurred. However, dengue is endemic in the city is evidenced by the fact that in two subsequent post-epidemic years dengue prevailed, though at low level and there was not much difference between these two years as far as percentages of dengue cases were concerned, though flow of fever cases as well as number of dengue cases was very much reduced in the third year of the study period, which also indirectly indicated the limited activity of the virus. In 2006, relatively much more cases were screened than that in the next year, probably due to alertness following the year of epidemic.

Such type of study, conducted perennially may give some idea about the intensity of the disease, the advent, seasonal incidence, waxing, and warning may also be monitored. When the cases begin to rise enormously and abruptly in the transmission season an impending epidemic may be apprehended (as it occurred in 2005), and appropriate measures can be adopted at proper time, avoiding any delay. As this study has generated some preliminary data, a comparative study between the years is also possible so far as the intensity and spread of the disease is concerned. From the proportion primary and secondary dengue cases, an idea of precipitation of DHF may also be assumed. It may be said that as the number of secondary dengue cases is much more than that of primary dengue cases in the present study, chance of occurring DHF remains in the community.

This is just a preliminary study. A large-scale active longitudinal serosurveillance with appropriate sample size and along with the study of vectorial capacity and competence, which is quite feasible, would provide more correct information.

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