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Trend of dengue virus infection at Lucknow, north India (2008- 2010): a hospital based study

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***Background & objectives*: Dengue virus (DV) infection has emerged as a major health problem in north India. Here, we report the annual trend of dengue virus infection as seen in Lucknow, Uttar Pradesh, during 2008-2010.**

***Methods*: Blood samples from clinically suspected cases of dengue virus infection were collected and history was taken on structured clinical data sheet. All samples were tested for dengue IgM by antibody capture ELISA. Selected samples were tested by conventional RT-PCR for dengue virus RNA. Weather information was continuously recorded from website of world weather information service.**

***Results*: There was a gradual increase in number of dengue fever cases with increased occurrence in 2010. Cases referred in January - December 2008 were 398 (54.5% anti DV IgM positive), in January - December 2009 were 599 (51.9% anti DV IgM positive) and in January - December 2010 were 1602 (64.9% anti DV IgM positive). Serotypes circulating in years 2008, 2009 and 2010 were DV-2 & DV-3, DV -1, 2 & 3 and DV-1 and DV-2 respectively. There is no statistical significant correlation between weather data and increasing dengue positive cases.**

***Interpretation & conclusions*: Increased cases of dengue fever were seen in 2010, which was not correlated with any change in environmental factors. A change in circulating serotypes was noted.**

Key words Annual trend - dengue virus infection - epidemic - outbreak - seasonality

Dengue fever (DF) has emerged as one of the world's major infectious diseases. Epidemics of dengue fever were first reported from the coastal area of Africa and later from Malaysia in the 19th century[1]. The infection is by now seen as a global epidemic with recorded prevalence in more than 120 countries[2]. Dengue is transmitted by *Aedes* mosquitoes, particularly *Aedes aegyptii* and, less important, *Ae. albopictus*. During the last 200 years, spread of the disease has increased, reaching endemic proportions during the last three decades[2].

In India, the first virologically confirmed epidemic occurred in Calcutta (now Kolkata) and the eastern coast of India in 1963-1964[3]. According to published reports all four serotypes of the virus are co-circulating in India[4]. A major widespread epidemic of dengue

haemorrhagic fever (DHF) occurred in 1996 involving areas around Delhi and Lucknow[5,6], since then, there has been a remarkable resurgence of the infection in north Indian plains that include the State of Uttar Pradesh. Once considered an urban problem, it has now penetrated into rural areas as well, due to high population density and other factors[7]. The illness occurs throughout the year with a peak during monsoon and post-monsoon season due to high vector density. Major outbreaks have occurred in north India[8]. Here we report the annual trend of dengue virus infection as seen in Lucknow, Uttar Pradesh, India, during the three years 2008-2010.

Material & Methods

Study area: Lucknow is situated at 26.75^0 latitude, 88.88^0 longitudes and 128 meter altitude in heart of Gangatic plain, has population of 2,750,447 and population density of 2011 person/km^2 (*http://www.lucknow.nic.in*). Climate is humid subtropical with cool dry winter from December to February and dry hot summer from April to June. The rainy season is from mid June to mid September. Average rainfall is 101 mm mostly from south west monsoon. Weather data were obtained from the website of world weather information service (*http://world wealther.wmo.int/*).

Laboratory methods: This study was conducted in the Department of Microbiology, King George Medical University (KGMU), Lucknow, over a period of 3 yr from 2008 to 2010. The hospital has total 2424 beds in different departments. Annually about 5,10,000 patients visit outpatient department (OPD) and 51,000 patients get indoor admission. Data from patients with suspected diagnosis of dengue virus infection, referred to us for laboratory confirmation, since January 2008 to December 2010, were analyzed. No intervention was done for the present study. The study protocol was approved by the institutional ethics committee and informed consent for using the patient sample and data was taken. Blood samples (5 ml) were collected and tested for anti DV IgM. Remaining sample was stored at -80^{o}C. Dengue virus specific IgM was tested by antibody capture ELISA (Mac ELISA) using commercial kits (Panbio, Australia). A detailed clinical history of all the cases was taken using a structured clinical data sheet.

Samples stored at -80^o C, >500 µl in volume and from patients having illness less than 7 days, were selected. These samples were tested by nested RT-PCR for serotype specific DV RNA. RNA was extracted from 250 µl serum samples by using Tri reagent [Sigma-Aldrich, USA] according to manufacturer's instructions. The identification was carried out by RT-PCR followed by nested PCR by demonstrating the presence of virus specific RNA employing dengue group-specific as well as serotype-specific primers targeting C-prM gene junction following the protocol of Lanciotti *et al*[9]. Negative (double distilled water) and positive controls (dengue virus culture fluid) were included at all the steps. Correlation between weather data and positive dengue cases was drawn using STATA 11 software (Texas, USA). Annual pattern of dengue infection was calculated by taking month-wise ratio of different variables in each year.

Results

A total of 398 cases were referred for diagnosis of dengue fever during January - December 2008, of which 217 (54.5 %) were anti DV IgM positive, during January - December 2009 there were 599 cases, of which 311 (51.9 %) were anti DV IgM positive, and during January - December 2010 there were 1602 cases, of which 1039 (64.9%) were positive for anti DV IgM antibody. Month-wise distribution of cases is given in Fig. 1. Age-wise distribution of total referred cases and anti DV IgM positive cases is shown in Table I. Paediatric references were more than adults, but in 2010 along with paediatric population adult references were also very high. Positivity rate among age groups was similar throughout the study (Table I). Male references were more than females, a usual trend seen in our total hospital admissions during study (data not shown).

Cases of dengue fever started increasing in the last week of June or early July every year and reached at peak during August to October (Fig. 1). Rainy season in Lucknow starts around late June and lasts till October. A seasonal peak of DV infection was recorded around 4-6 wk after arrival of rain every year. There was no strong statistical evidence of increase in anti DV IgM positive cases in 2010, with change in maximum temperature (R= 0.0135, P=0.937), minimum temperature (R=0.1550, P=0.3668), humidity (R= 0.3077, P=0.0679) and amount of rain fall (R=0.0431, P=0.8029) (Fig. 2).

Selected serum samples were tested for detection of DV RNA. In 2008 only 42 samples were tested and DV-2 and DV-3 serotypes were detected in 10 and 8 samples, respectively. In 2009, a total of 69 samples were tested and DV-1 was detected in 5 samples, DV-2

Table I. Age-wise distribution of total referred and anti DV IgM positive cases

Year	2008		2009		2010	
Age group (yr)	Total	Positive (%)	Total	Positive (%)	Total	Positive (%)
0-5	150	85 (56.67)	253	130 (51.3)	310	218 (70.3)
>5-15	163	91 (55.8)	260	147 (56.5)	276	182 (59.4)
>15-25	034	18 (52.9)	48	14 (29.1)	424	270 (63.6)
>25-35	21	11 (52.3)	17	8 (47.1)	325	210 (64..6)
>35-45	11	5 (45.4)	10	6	152	85 (55.9)
>45-55	9	4 (44.4)	5	3	65	38 (58.4)
>55-65	8	3	4	2	32	23
>65	2	0	2	1	18	13
Total	398	217 (54.5)	599	311 (51.9)	1602	1039 (64.9)

in 20 samples, and DV-3 in 15 samples. In 2010, of the 80 serum samples tested, DV-1 was detected in 16 samples and DV-2 was detected in 4 samples. DV-4 was not detected throughout.

Discussion

During 2008-2010, an increasing number of dengue virus infection cases was observed, reaching an epidemic proportion in 2010, with change in circulating serotype. Dengue fever is an ancient disease. Dengue has emerged as a major health problem in north Indian region. The epidemiology of dengue virus and its prevalent serotypes keep on changing. In India, different circulating serotypes have been reported during different outbreaks (Table II). In 2006 all the four serotypes were co-circulating in north Indian plains. DV-2 and DV-3 were the dominant serotypes during 2008-2009, which were also circulating in previous years. In 2009, DV-1 appeared and in 2010 it became the dominant serotype.

Rain, temperature and relative humidity are reported as the major and important climatic factors, which could alone or collectively be responsible for an epidemic. In north India, the largest proportion of serologically positive cases have been recorded in the post-monsoon period[27]. Our findings were similar to those reported by other groups from this geographical region[4,5]. In a study done in Bangladesh, the seasonal occurrence of

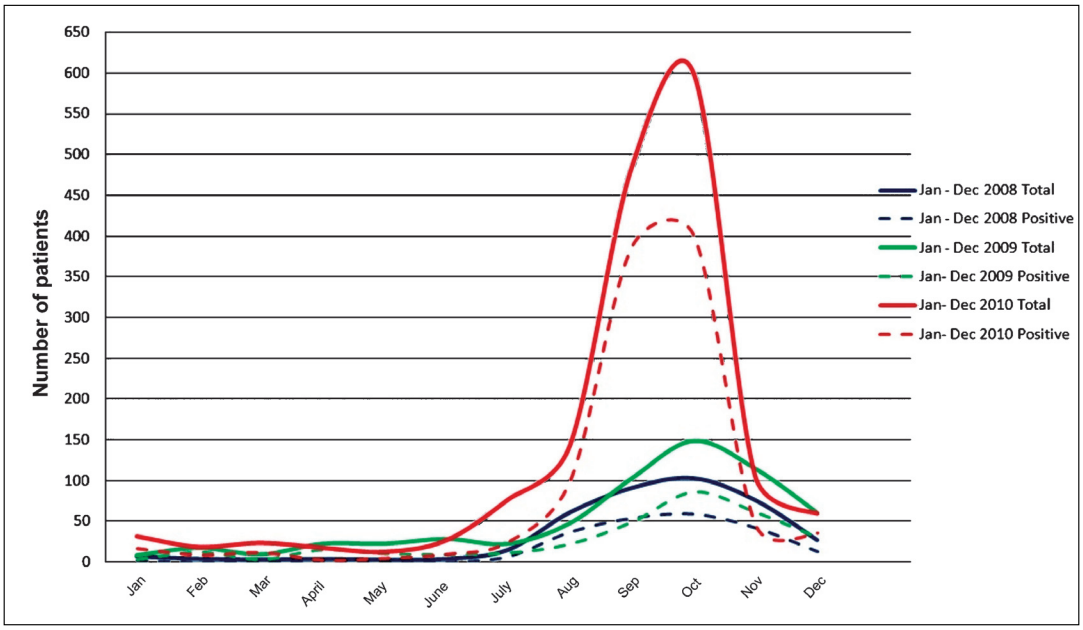

Fig. 1. Month-wise distribution of total suspected and anti DV positive cases in different years.

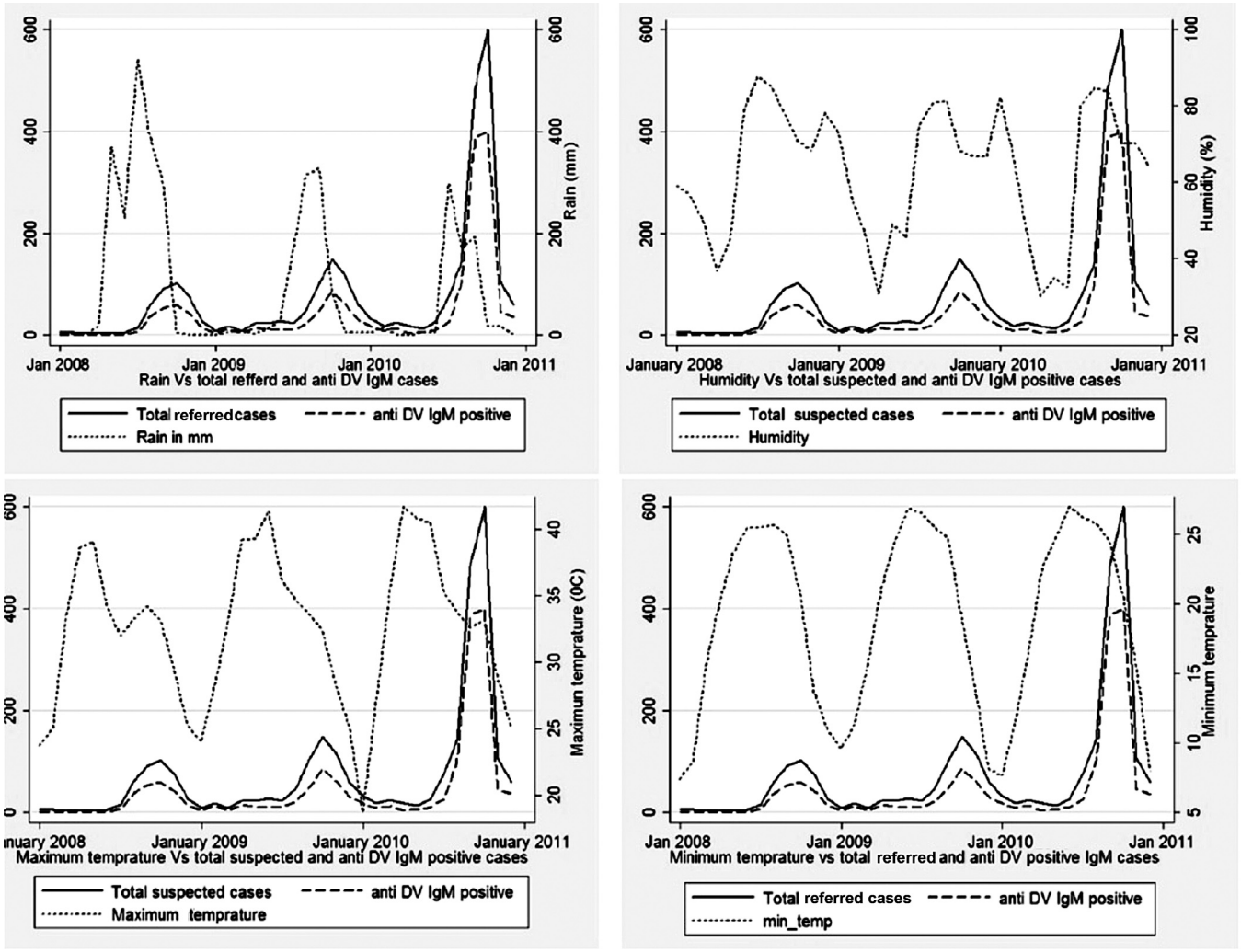

Fig. 2. Correlation of rain fall, humidity, maximum temperature, minimum temperature, with total referred dengure cases and anti DV IgM positive cases.

positive cases has shown that post-monsoon period is the most affected period[28]. Studies have proposed that ecological and climatic factors influence the seasonal prevalence of the vector *Ae. aegypti* and dengue virus[29]. The vector has adapted to extremes of warm and cold weather resulting in occurrence of dengue cases round the year. Dengue virus and the vector have been reported in the arid zones of Rajasthan as well[17].

In 2008 and 2009 the highest number of cases that were positive for anti DV IgM were from paediatrics population. In 2010, a shift was seen toward higher age group. In Asian countries where dengue has been epidemic for several years, this age shift is clearly observed, indicating an epidemiological change in dengue infection[30].

This is a hospital based study and may not represent the true community picture as only highly suspected or confirmed cases are reported here. Increase in awareness, better diagnostic facilities, availability of more sensitive and specific diagnostic tests can influence reporting pattern to some extent.

In conclusion, an increase in DF cases was seen in 2010 compared to previous two years, and this increase was not related to changes in environmental factors. DV-1 was found to be the dominant serotype in 2010. Contentious efforts for long term laboratory based surveillance systems should be made that can forecast dengue epidemics. This will help alert the public and physicians to diagnose and properly treat DF/DHF cases.

Table II. Epidemiology of dengue circulating serotype in India

Year	Site in India	Virus serotype	References
1965	Ajmer (Rajasthan)	DV-1 and DV-3	10
1966	Vellore (Karnataka)	DV-3	11,12
1966	Jabalpur (Madhya Pradesh)	DV-3	13
1968	Vellore (Karnataka)	DV-1, Dv-2, DV-3, DV-4	14
1970	Hardoi (Uttar Pradesh)	DV-2	15
1983	Calcutta (now Kolkata) (West Bengal)	DV-3	16
1985	Jalore (Rajasthan)	DV-3	17
1988, 1989	Surat, Rajkot, Sabarkantha (Gujrat)	DV-2	18
1990	Calcutta (now Kolkata) (West Bengal)	DV-3	19
1993	Jammu (Jammu and Kasmir)	DV-2	20
1996	Hissar (Haryana)	DV-2	21
1996	Delhi	DV-2	5
1996	Lucknow (Uttar Pradesh)	DV-2	6
1997	Delhi	DV-1	22
2001	Gwalior (Madhya Pradesh)	DV-2	23
2003	Gwalior (Madhya Pradesh)	DV-3	8
2004	Gwalior (Madhya Pradesh)	DV-3	24
2005	Delhi	DV-3	25
2006	Delhi	DV-1, DV-3	26
2006	Delhi	DV-1, DV-2, DV-3, DV-4, concurrent infection with all serotypes.	4

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