

A Hospital Based Serosurveillance Study of Dengue Infection in Jaipur (Rajasthan) , India

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ABSTRACT

Background: Dengue has been known to be endemic in India for over two centuries. There is a need to assess the magnitude of dengue virus establishment in the state of Rajasthan. A surveillance study was conducted to analyze dengue seropositivity among patients with clinical suspicion of dengue fever like illness, who presented to or were admitted at a tertiary care private hospital at Jaipur.

Methods: Serum samples from 2169 suspected dengue cases (1356 males and 813 females) were received in the Serology lab over the four year study period (2008-2011). The samples were subjected to a rapid immuno-chromatography assay with differential detection of IgM and IgG antibodies. A primary dengue infection was defined by a positive IgM band and a negative IgG

band, whereas a secondary infection was defined by a positive IgG band with or without an IgM band.

Result: Among the 2169 patients who were screened; 18.99% (412) were dengue specific IgM positive cases. 64.49% (1399) cases were negative for dengue specific antibodies, 5.67% (123) were primary dengue cases, and 23.51% (510) were total secondary dengue cases. During the study period, the Dengue IgM seropositivity was highest in the year 2009 and was lowest in the year 2011. Most of the cases occurred in the post-monsoon season, with a peak in the month of October, each year.

Conclusion: A detailed and continuous epidemiological surveillance is required, for monitoring the incursion and spread of dengue viruses. This will help in undertaking and implementing effective control and management strategies.

Key words: Dengue, Serosurveillance, Jaipur

INTRODUCTION

Dengue is the most important arthropod – borne viral infection of humans. Globally, an estimated 2.5 billion people are at a risk of Dengue, and approximately 975 million of these live in urban areas of the tropical and sub-tropical countries of south east Asia, the Pacific and the Americas [1]. Each year, 50-100 million cases occur, hospitalizations for the infection have reached 500,000 and the global death toll is >20,000 persons [2].

Dengue virus belongs to the *Flaviviridae* family and it is maintained in nature primarily through a biological transmission between susceptible vertebrate hosts by haematophagous arthropods [3]. The principle vector is a day biting, domestic mosquito, *Aedes aegypti*; although a second dengue vector, *Aedes albopictus*, has also been implicated for dengue transmission in Asia [4].

The dengue virus has antigenically four distinct serotypes which are called DEN-1, DEN-2, DEN-3 and DEN-4. Each serotype of the virus produces a specific, lifelong immunity, but it provides only a short term cross-immunity [5]. Dengue virus infection produces a spectrum of clinical illnesses which range from asymptomatic or mild febrile illnesses, to classic Dengue Fever (DF), to the most severe form of illness, dengue haemorrhagic fever (DHF) [6]. Classic dengue fever is marked by a rapid onset of high fever, headache, retro-orbital pain, diffuse body pain (both muscle and bone), weakness, vomiting, sore throat, an altered taste sensation, and a centrifugal maculopapular rash, among other manifestations. DHF and DHS are severe potentially fatal complications which are often associated with an infection by a second serotype [7].

The first epidemic of a clinical dengue like illness in India was recorded in Chennai (Madras) in 1780 [8]. In the past decade, dengue has assumed pan-India proportions. Among 18 endemic states/UTs, the most affected regions are Delhi, West Bengal, Kerala, Tamil Nadu, Karnataka, Maharashtra, Rajasthan, Gujarat and Haryana.

In fact, the dengue case fatality rate has been above 1% over the last 10 years [9]. According to the estimates of National Vector Borne Disease Control Programme, 47,209 dengue cases were reported in India in the year 2012 [10]. A recent study from New Delhi reported that all four dengue serotypes were detected in 2003, whereas the data of 2004, 2005 and 2006 have revealed the predominance of DEN-3. Predominance of DEN-2 was observed in the year 2007, whereas in 2008, DEN-1 was the most common serotype isolated [11]. Unplanned urbanization and migration of populations from rural to urban areas, with lack of proper sanitation facilities, are important factors which have resulted in an increased burden of dengue in recent times in India [12].

This is a four year, hospital based serosurveillance study, conducted to assess the magnitude of Dengue virus problem in Jaipur by analyzing the seropositivity of dengue virus specific antibodies in patients with suspected dengue fever like illness.

MATERIAL AND METHODS

This serosurveillance study was conducted on the serum specimens which were received in the Serology lab for detection of dengue IgM and IgG antibodies, from patients with clinical suspicion of dengue fever like illness, who presented to the outpatients department or were admitted to a tertiary care private hospital in Jaipur, Rajasthan between January 2008-December 2011. A total of 2169 consecutive, non-repetitive serum samples were received from suspected dengue cases (1356 males and 813 females) during the study period. The samples were usually collected 5-10 days following the onset of illness, but the exact date of sampling post fever was not available for most of the patients.

The samples were processed in lab by using a rapid Immuno-chromatography (ICT) based kit, with a differential detection of IgM and IgG antibodies. The results were graded as reactive (visible band)

or non reactive (no band). The rapid ICT is a qualitative membrane based immunoassay for the detection of dengue specific antibodies in whole blood, serum or plasma. When they are present in the patients' samples, dengue specific IgM or IgG antibodies bind to anti-human IgM or IgG antibodies which are immobilized in two lines across the cassette membrane. Colloidal gold complexes which contain recombinant dengue 1-4 antigens are captured by the bound patient IgM or IgG to give visible pink line(s). A procedural control was included in the assay, to indicate that it had been performed correctly. The IgG cut off in the test was set to detect high IgG levels which were characteristic of secondary infections and therefore, the primary dengue infection was defined by a visible IgM band without a visible IgG band, whereas a secondary infection was defined by a positive IgG band with/without a positive IgM band [13].

RESULTS

During the study period (2008-2011), the total number of samples which was screened for Dengue specific IgM and IgG antibodies was 2169. The age and sex distribution of suspected dengue cases has been shown in [Table/Fig-1]. The dengue serology samples were received from 62.52% (1356) males and 37.48% (813) females, and from 19.27% (418) children (<15 years) and 80.73% (1751) adults (>15 years).

[Table/Fig-2] shows the year wise distribution of suspected dengue cases and dengue positive cases. Of the 2169 samples which were tested, 18.99% (412) were dengue positive cases i.e. positive for dengue virus specific IgM antibodies. The highest number of samples was tested in the lab for dengue serology in the year 2010. However, the percentage of IgM positivity was highest in 2009 and it was the least in 2011.

[Table/Fig-3] shows the age and sex distribution of Dengue positive cases. Among the 2169 samples which were screened (1356 males and 813 females), the IgM positivity was found to be 292 (21.53%) for males and it was 120 (14.76%) for females. Among 412 dengue IgM seropositive patients, 70.87% (292) were males and 29.12% (120) were females. Maximum dengue specific IgM positivity was noted in both males and females who were in the age group of 16-30 years.

[Table/Fig-4] is the representation of the Serological pattern of dengue infection in Jaipur. Among the 2169 patients who were screened; 18.99% (412) were total dengue positive cases (i.e.

| Year/Total No. of Sera of Dengue Suspected Cases Tested | No. of Males <15 Years | No. of Males >15 Years | No. of Females <15 Yrs | No. of Females >15 Yrs |
|---|------------------------|------------------------|------------------------|------------------------|
| 2008/260 | 47 | 113 | 31 | 69 |
| 2009/551 | 63 | 274 | 39 | 175 |
| 2010/754 | 73 | 387 | 51 | 243 |
| 2011/604 | 77 | 322 | 37 | 168 |
| Total 2169 | 260 | 1096 | 158 | 655 |
| % | 11.98% | 50.53% | 7.28% | 30.19% |

[Table/Fig-1]: Age and Sex Distribution of Suspected Dengue Patients

| Year | Total No. of Suspected Dengue Cases | No. of Total Dengue Positive Cases (IgM +) |
|-------|-------------------------------------|--|
| 2008 | 260 | 55(21.15%) |
| 2009 | 551 | 119(21.59%) |
| 2010 | 754 | 142(18.83%) |
| 2011 | 604 | 96(15.89%) |
| Total | 2169 | 412(18.99%) |

[Table/Fig-2]: Year Wise Distribution of Suspected Dengue Fever Cases and Dengue Positive Cases

dengue specific IgM positive). 64.49% (1399) were not sufferers of dengue, 5.67% (123) were primary dengue cases, and 23.51% (510) were total secondary dengue cases, which included 10.18% (221) old dengue cases and 10.09% (61) true secondary dengue cases.

[Table/Fig-5] shows the month wise analysis of dengue suspected cases and dengue positive cases. Maximum number of dengue cases occurred in the post-monsoon season, with a peak in the month of October each year.

DISCUSSION

Dengue is an important, emerging disease of the tropical and sub-tropical regions today. It has been known to be endemic in India for over two centuries, as a benign and a self limited disease. No study from this part of the country i.e. the state of Rajasthan has been conducted till date, to analyze the seroprevalence of dengue specific antibodies from patients with clinically suspected dengue fever. However, clinical and virological studies which were done on outbreak investigations of dengue fever in Ajmer city (in 1969) and in Jalore town (in 1985) have been previously reported in literature [14,15]. Another recent study has described the distribution of dengue virus types in *Aedes aegypti* and it has reported DEN-3 in urban settings and all 4 DEN types among rural settings of semiarid region (Jaipur) in Rajasthan [16].

Laboratory diagnosis of dengue infection is crucial, as the broad spectrum of clinical presentations can make accurate diagnosis difficult. Among the methods which are available for diagnosis of dengue, virus isolation provides the most specific test results. However, facilities that can support viral culture are not always available. The detection of viral genome and viral antigens also provides an evidence of infection. Seroconversion of IgM or IgG antibodies is the standard for serologically confirming a dengue infection [1]. Dengue virus specific IgM antibodies tend to appear as early as 3 days after infection and remains in circulation for 30-60 days. IgG antibodies arise at about 7 days, they reach a peak at 2-3 weeks and persist for life. Detection of dengue specific IgM antibodies allows a provisional diagnosis to be made from a single serum sample [17].

In this study, 18.99% patients had serologically confirmed dengue infection. In conformation to our findings, Garg et al., reported the seroprevalence of dengue infection at a teaching hospital in Kanpur, northern India, to be 19.7% [6]. A recent study which was conducted on 8138 samples at a Government hospital in Delhi, reported that 19.66% (1600) samples were positive for dengue specific IgM [18]. However, another study from New Delhi, which was done on 1820 serum samples from suspected dengue cases, reported that 44.56% samples were confirmed for dengue infection serologically [19]. A study from central India reported 31.3% patients to be serologically positive for dengue infection [20].

A higher prevalence of dengue infection was noted in males than in females. The male to female ratio in this study was 2.43:1. This may be the representation of those who visited the hospital to seek care rather the truly infected population.

The age group which was most affected in this study was the 16-30 years age group. Our findings were contrary to those of some Indian studies which had reported the vulnerability of children to dengue infection [6, 17, 21]. However, a few hospital based studies have similarly reported increasing infection rates among adults [22-24].

On the basis of the data which was collected, the population of this study could be categorized into several distinct groups: Among the 2169 patients who were screened; 18.99% (412) were total dengue positive cases (i.e. dengue specific IgM positive). In the study 64.49% (1399) were not sufferers of dengue, 5.67% (123)

| Age (in years) | Males with Primary Dengue Infection (With Only Dengue IgM +) | Males with True Secondary Dengue Infection (Both Dengue IgM & IgG +) | Total Dengue Positive Males | Females with Primary Dengue Infection (with Only Dengue IgM +) | Females with True Secondary Dengue Infection (Both Dengue IgM & IgG +) | Total Dengue Positive Females |
|----------------|--|--|-----------------------------|--|--|-------------------------------|
| 0-15 | 23 | 34 | 57(19.52) | 19 | 16 | 36(30%) |
| 16-30 | 20 | 120 | 140(47.94) | 20 | 25 | 45(37.50%) |
| 31-45 | 15 | 36 | 52(17.80) | 3 | 22 | 25(20.83%) |
| 46-60 | 11 | 15 | 26(8.9) | 3 | 10 | 13(10.83%) |
| 61-75 | 7 | 7 | 13(4.45%) | 0 | 2 | 2(1.66%) |
| 76-90 | 2 | 1 | 3(1.02%) | 0 | 0 | 0(0) |
| >90 | 0 | 1 | 1(0.34%) | 0 | 0 | 0(0) |
| Total | 78 | 214 | 292 (70.87%) | 45 | 75 | 120(29.12%) |

[Table/Fig-3]: Age and Sex Distribution of Patients Suffering with Primary and Secondary Dengue Infections

| Year/No. of Sera of Suspected Dengue Cases Tested | No of Pts. Having No Dengue Antibody (No Dengue Infection) | No of Pts. Having IgG Antibody Only (Old Dengue Cases) | No of Pts. Having Either IgM | No of Pts. Having Igm Antibody Alone (Primary Dengue Cases) | No. of Pts. Having Either IgG or IgG with IgM Antibodies | No. of Pts Having both IgG and IgM Antibodies (True Secondary Cases) |
|---|--|--|--|---|--|--|
| | | | Or IgM and IgG Antibodies (Total Dengue Cases) | | (Total Secondary Dengue Cases) | |
| Year 2008/260 | 154 (59.23%) | 42 (16.15%) | 55 (21.15%) | 29 (11.15) | 68 (13.33%) | 26 (10%) |
| Year 2009/551 | 300 (54.44%) | 78 (14.15%) | 119 (21.59%) | 21 (3.81%) | 176 (34.50%) | 98 (17.78%) |
| Year 2010/754 | 496 (65.78%) | 70 (9.28%) | 142 (18.83%) | 38 (5.03%) | 174 (34.11%) | 104 (13.73%) |
| Year 2011/604 | 449 (67.71%) | 31 (5.13%) | 96 (15.89%) | 35 (5.79%) | 92 (18.03%) | 61 (10.09%) |
| Total 2169 | 1399 (64.49%) | 221 (10.18%) | 412 (18.99%) | 123 (5.67%) | 510 (23.51%) | 289 (13.32%) |

[Table/Fig-4]: Serological Studies on Dengue in Jaipur (2008-2011)

| Year | 2008 | 2008 | 2009 | 2009 | 2010 | 2010 | 2011 | 2011 | | |
|-------|-------------|------------------------|-------------|------------------------|-------------|------------------------|-------------|------------------------|-------------------|------------------------------|
| Month | Sera Tested | Sera with Dengue IgM + | Sera Tested | Sera with Dengue IgM + | Sera Tested | Sera with Dengue IgM + | Sera Tested | Sera with Dengue IgM + | Total Sera Tested | Total Sera With Dengue IgM + |
| Jan | 0 | 0 | 8 | 0 | 12 | 1 | 9 | 0 | 29 | 1 (3.44%) |
| Feb | 4 | 0 | 4 | 1 | 5 | 0 | 12 | 2 | 25 | 3 (12%) |
| March | 4 | 1 | 14 | 0 | 8 | 0 | 13 | 0 | 39 | 1 (2.56%) |
| April | 1 | 0 | 20 | 2 | 16 | 3 | 24 | 1 | 61 | 6 (9.83%) |
| May | 1 | 0 | 14 | 1 | 18 | 1 | 37 | 0 | 70 | 2 (2.85%) |
| June | 3 | 1 | 8 | 2 | 10 | 1 | 31 | 1 | 52 | 5 (9.61%) |
| July | 8 | 4 | 16 | 1 | 9 | 2 | 18 | 0 | 51 | 7 (13.72%) |
| Aug | 17 | 3 | 29 | 9 | 28 | 4 | 44 | 2 | 118 | 18 (15.25%) |
| Sept | 85 | 16 | 103 | 21 | 116 | 21 | 85 | 13 | 389 | 71 (18.25%) |
| Oct | 80 | 20 | 220 | 59 | 293 | 62 | 158 | 41 | 751 | 182 (24.23%) |
| Nov | 40 | 6 | 92 | 22 | 210 | 44 | 131 | 30 | 473 | 102 (21.56%) |
| Dec | 17 | 4 | 23 | 1 | 29 | 3 | 42 | 6 | 111 | 14 (12.61%) |
| Total | 260 | 55 | 551 | 119 | 754 | 142 | 604 | 96 | 2169 | 412 (18.99%) |

[Table/Fig-5]: Month wise Analysis of Dengue Cases

were primary dengue cases with only IgM antibodies, and 23.51% (510) were total secondary dengue cases. These included 10.18% (221) old dengue cases with only IgG antibodies and 10.09% (61) true secondary dengue cases with both IgG and IgM antibodies.

A monthwise analysis of dengue infections revealed that dengue cases increased in number gradually from July onwards and that they peaked in the month of October each year. This seasonality of transmission of dengue, with an increased activity post monsoon, was in accordance with reported patterns of dengue transmission

[25-27]. The presence of stagnating water after rainfall favours breeding of the mosquito vector, resulting in an increase in dengue cases. Hence, it is recommended that preventive measures should be implemented during the monsoon and post monsoon months.

CONCLUSION

This study showed a significant prevalence of dengue infection among suspected dengue patients and it hence reflects that dengue is fast emerging as a major health concern in Jaipur. Involvement of many laboratories in diagnosis of dengue, coupled

with general awareness among the public and constant vigilance by healthcare officials, is needed in combating dengue. Further studies are required to map out the prevalence of different serotypes and genotypes of dengue viruses in Rajasthan, so as to forecast any future outbreak of DHF in the state.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Jan 04, 2013**
Date of Peer Review: **May 17, 2013**
Date of Acceptance: **Jun 23, 2013**
Date of Publishing: **Sept 10, 2013**