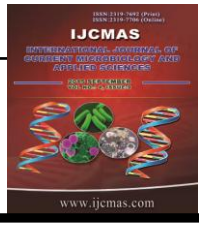
Int.J.Curr.Microbiol.App.Sci (2015) 4(9): 933-940

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 9 (2015) pp. 933-940**
http://www.ijcmas.com



Original Research Article

Seroprevalence of Dengue in Central Rajasthan: A Study at a Tertiary Care Hospital

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Keywords

Dengue, Seroprevalence, IgG & IgM Antibody, Dengue Day1 rapid test, Ajmer, Vector

A B S T R A C T

Dengue fever is an acute febrile Arbo-viral disease affecting the tropical and subtropical regions of the world. Dengue is endemic to the Indian sub-continent and it is associated with explosive urban epidemics and has become a major public health problem in India. It is a notifiable disease, but its exact prevalence is difficult to quantify due to the frequency of epidemics which appear throughout the country. This study was conducted to report the prevalence of Dengue virus infection in Ajmer. Study was performed at a tertiary care hospital in Ajmer, Rajasthan in year 2015. Patients attending hospitals across Ajmer for suspected dengue were tested. Blood samples collected in plain tubes were tested for dengue IgM & IgG antibodies, NS1 antigen by Dengue Day 1 test respectively. The laboratory records were analyzed for demographic features and seasonal variations. Descriptive statistics were used. Data were expressed in proportions. Out of total 10706 serum samples tested, 380 were found positive for dengue virus infection. 62.63% positive samples were of male patients and 31.58 positive samples were from 10 to 20 years age group. Seasonal trend showed a gradual increase in dengue positives started from August with a peak in October (6.07%). The most common presentation was fever (100%) while only 4% cases presented CNS Symptoms. Dengue has established its transmission in urban and semi-urban areas of Ajmer with predominantly affecting males. Virus activity is high during monsoon and post monsoon period which coincides with increased vector breeding. This study thus emphasizes the need for continuous sero epidemiological surveillance for the timely formulation and implementation of effective dengue control programme.

Introduction

Dengue fever is an acute febrile Arbo-viral disease affecting the tropical and subtropical regions of the world[1].

The name 'Dengue' is derived from the Swahili word *'Ki denga pepo',* which means 'sudden seizure by the demon'. Following the Philadelphia epidemic in 1780, it was called as the 'break bone fever' by Benjamin Rush[2].

The dengue virus is an arthropod borne

virus-*Arbovirus*, belonging to the family Flaviviridae and genus Flavivirus. It is a mosquito borne (arthropod) viral infection and is transmitted, primarily by Aedes aegypti and sometimes by Aedes albopictus[3,4].

Dengue is caused by four distinct serotypes of viruses; DEN-1, DEN-2, DEN-3 and DEN-4[5].

Dengue virus causes a spectrum of illness ranging from in apparent, self limiting Classical dengue fever (DF) to life threatening Dengue hemorrhagic fever (DHF) and Dengue shock syndrome (DSS)[6].WHO estimates there are 50–100 million dengue infections worldwide every year. 6.3 million Children under age five died in 2013, nearly 17 000 die every day[7].

Dengue is endemic to the Indian sub-continent. Dengue is associated with explosive urban epidemics and has become a major public health problem in India.

In India, a dengue virus infection has been frequently encountered in epidemic proportions in several states[8-10] It is a notifiable disease, but its exact prevalence is difficult to quantify due to the frequency of epidemics which appear throughout the country. Although dengue serotype 2 is the most prevalent serotype over the past 50 years, serotypes 3 and 4 have appeared in some epidemics[1, 11-14]. Primary DENV infections present as either a non-specific illness or dengue fever (DF). Secondary infection with a serotype different from that causing primary infection may lead to dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS)[3].

Dengue is an enveloped virus with a single-stranded, positive sense RNA genome of about 11 kb containing a single open reading frame enconding a single polyprotein co- and post translationally cleaved into 3 structural (C, prM and E) and 7 nonstructural proteins (NS1, NS2A, NS2B, NS, NS4A, NS4B and NS5)[5] .There is no specific treatment for dengue/ severe dengue, but early detection and access to proper medical care lowers fatality rates below 1%[7].

The objectives of this study were to know the demographic characteristics of cases; to understand the seasonal trend and pattern of disease; and to study disease outcome in dengue.

Materials and Methods

The study was conducted in the Department of Microbiology, J.L.N. Medical College and associated groups of hospital, Ajmer from the period of July 2014 to June 2015.

Patients enrolled in the study were from the following hospitals,

1) J.L.N Medical Hospital
2) Zanana Hospital and associated groups of hospital, Ajmer.

The samples were subjected to Dengue Day 1 Rapid test

Method of collection of data

Inclusion Criteria

Individuals attending outpatient departments and inpatients of J.L.N Medical college and associated group of Hospital, Ajmer, with symptoms of fever for more than five days duration, with more than or equal to two of the following;

1) Joint pain,
2) Rash,

3) Myalgia,
4) Retro-orbital pain,
5) Headache and
6) Haemorrhagic manifestation.

Specimen collection

About 3-5ml blood was collected in a sterile vial with all aseptic precautions.

1) Half of the blood was allowed to clot at room temperature for half an hour, after which the clot was dislodged to separate the serum. This was centrifuged at 3000rpm (rotations per minute) for five minutes.
2) To the other half of blood, EDTA was added and this anticoagulated blood was sent for hematocrit and platelet count estimation.

Test performed

RAPID TEST (Dengue Day 1 test)

Dengue Day 1 rapid test is an *in vitro* immunochromatographic, one step assay designed to detect IgM and IgG antibodies to dengue virus in human serum & NS-1 antigen.

Dengue Day 1 Test kit consists two devices; one device for detection of Dengue NS 1 antigen and second device for the differential detection of Dengue IgM/IgG antibodies in human serum/plasma. Dengue NS 1 antigen device contains two lines; "C" (control line) & "T"(Dengue NS 1 Antigen detection Test Line). Test line is coated with antibodies, anti-dengue NS 1 Ag. When sample is added to the device, Dengue NS 1 antigen, if present in the sample will bind to the anti-dengue NS 1 gold colloid conjugates making antigen-antibody complex. This complex migrates along the membrane to the test region and forms the visible pink line at T as antibody-antigen-antibody gold colloid forms.

Dengue IgM/IgG contains three lines "C" (control line), "M" (IgM test line), "g" (IgG Test Line). IgM Test line is coated with anti-human IgM and Igg test line is coated with anti-human IgG. When sample is added to the device, IgM and IgG antibodies in the sample react with anti-human IgM or IgG antibodies coated on the membrane respectively. Colloidal gold complex containing dengue 1–4 antigen is captured by the bound anti-dengue IgM or IgG on respective test bands located in the test window causing a pale to dark red band to form the IgG or IgM region of the test device window. The intensity of the test bands in the respective device will vary depending upon the amount of antigen/antibody present in the sample. The appearance of any pink/red in a specific test region should be considered positive for that particular antigen or/and antibody type (IgG or IgM). A red procedural control line should always develop in the test device window to indicate that the test has been performed properly.

Test procedure

1. Bring the required number of Dengue Day 1 Test foil pouches and specimen to room temperature prior to testing.
2. Remove the test card from the foil pouch prior to use.
3. Label the test card with patient's name or identification number.
4. Perform the test on both the devices as follows.

Dengue NS 1 Antigen device

i) Add 3 drops (100µl) of sample (serum/plasma) using Dengue Antigen Test sample dropper to the sample well of antigen device.
ii) Allow reaction to occur for 20 minutes.

iii) Read result at 20 minutes. Positive result may appear as early as 2-10 minutes. However, negative results must be confirmed after 20 minutes only.

B) Dengue IgM/IgG device

i) Fill the Dengue Antibody lower circular part of the sample dropper with the specimen upto the mark provided on the dropper. Then add the specimen to the sample well "S" of antibody device. This will add 10µl of specimen to the device. Dispose of the dropper considering it to be biohazardous. Alternatively, add 10µl of sample using micropipette to the sample well of the antibody device.

Interpretation of the Dengue day 1 rapid test

The presence of each one color line (control) within the result window indicates a negative result. The control line (C) and IgM line (M) are visible on the test device. This is positive for IgM antibodies to Dengue virus and indicates primary dengue infection.

The control line and IgG line (G) are visible on the test device. This is positive for IgG antibodies and indicates of secondary or past dengue infection. The control line, IgM line (M) and IgG line (G) are visible on the test device. This is positive for both IgM and IgG antibodies and indicates late primary or early secondary dengue infection. The control line, NS-1 Ag line is visible on the test device. This is positive for NS-1 antigen and indicates of early acute dengue infection.

Performance characteristics
Dengue NS 1 Ag

Sensitivity: 100% Specificity: 99.94%

Dengue IgM/IgG antibody

Sensitivity: 100% Specificity: 99.88%

As per the manual provided with Dengue DAY 1 Test kit

Results and Discussion

During the study period, a total of 10706 blood samples were tested for dengue. Of the total samples tested, only 3.55% (n=380) were found to be positive for dengue virus. Out of the 380 dengue positive cases, 136(35.79%) were NS-1 positive, 117(30.79%) were IgM positive, 38(10%) were IgG positive, 71(18.68%) were IgG/IgM positive, 14(3.68%) were IgG NS-1/IgMNS-1 positive and 4(1.05%) were IgGIgMNS-1 positive.

Of all the patients tested, 6423 were males and 4283 females. From the total positives for dengue, 62.63% (n=238) were males and 37.37% (n=142) females. So, it was observed that dengue affected males and females in a ratio of 3:2.

During this study, a majority of patients tested positive for dengue cases were of 10 to 20 yrs age group (n=120, 31.58%) followed by the age group 20 to 30 yrs (n=60, 15.78%).

In the study population, highest numbers of patients were tested for dengue in the month of November (n=1400) followed by October (n=1187) and December (n=988). A gradual increase in dengue positive cases was noticed from August (n=16) with a highest peak in October (n=72) and November (n=76) [Figure-2].

As analyzed from table no.2, fever was present in almost all cases (n=380) followed by, headache (n=274), joint pain (n=2432), myalgia (n=144), retro-orbital pain (n=141),

backache (n=95), skin rash (n=80). Hemorrhagic manifestations were present in 57 cases, while CNS symptoms were found in only 15 cases.

In this study, 3.55% patients were positive for dengue infection. These findings are in accordance with other studies conducted in India by A Garg[8] and R Paramasivan[10].

The higher prevalence of dengue infection was noted among males than females. The male to female ratio was 3:2 which correlates well with other studies. High prevalence amongst males is probably due to more outdoor activities by males in comparison to females which results in more exposure to day biting mosquitoes.

Table.1 Symptoms and Complications of Confirmed Dengue Cases (n=380)

Clinical symptoms	Dengue positive cases	Percentage
Fever	380	100
Headache	274	72
Joint – pain	243	64
Retro –orbital pain	141	37
Myalgia	144	38
Backache	95	25
Rash	80	21
Hemorrhage	57	15
CNS symptoms	15	4

Figure.1 Age wise Distribution of Dengue positive Cases during 2014-15

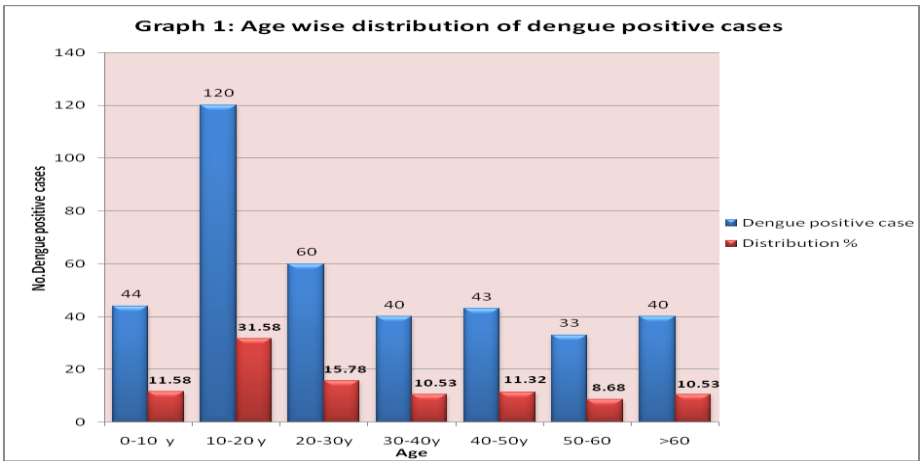
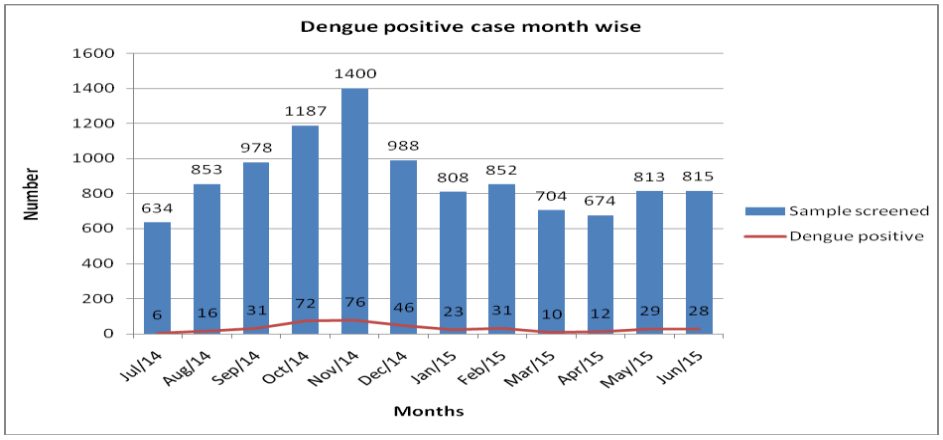


Figure.2 Month wise Distribution of Dengue Cases during 2014-15



The most common age group of infection in the present study was 10-20 years which includes the children and young adults. This was comparable to other studies of Gore MM, Baruah[15]and Dash PK et al[16]. The high number of cases in the paediatrics and young adult age group implies that the disease is endemic in these regions. In these areas, adults manifest with disease less, as they become immune to the virus.

However, in the study conducted by Neerja M et al[17], high numbers of cases were seen in the adult age group. This indicates that the virus had been introduced to a non-exposed population and disease was not endemic.

True endemicity will be reached when the adult infection declines and only the new entrants into the population, that is, the children, are affected more by the disease.

To identify the seasonal variation of the disease, analysis of the data on monthly basis was done. The infection started spreading in August, peaked in October and slowly tapered by December. The seasonality of transmission of dengue with increased activity in monsoon and post monsoon season was seen in the present study; in accordance with the reported patterns of dengue transmission[18]. This seasonal outbreak of disease transmission is very important at local level for effective control measures and that preventive measures should come into full swing during water stagnation periods after the initial bouts of rainfall and at the end of monsoon. The clinical profile of dengue revealed that fever was the most common presenting symptom, 380 (100%). Similar studies in and around India have also reported the same pattern[19].

In conclusion, the geographical spread of all four DENV serotypes throughout the subtropical regions of the world has led to larger and more severe outbreaks and the accurate and efficient diagnosis of the disease is important for clinical care, surveillance, pathogenesis studies and vaccine research. Furthermore, an efficient diagnosis is an important tool to support Epidemiological Surveillance Programs considering the difficulties in confirming dengue cases based only on the clinical symptoms, especially during inter-epidemic periods.

In view of the high mortality rate and to reduce the disease burden, it is imperative to have a rapid and sensitive laboratory assay for early detection of the disease. Dengue

affected predominantly males and paediatrics well as adult population. A seasonal trend was observed for dengue infections with maximum cases in post monsoon and late monsoon months which coincides with increased breeding of mosquitoes during these seasons. Therefore, vector control measures should be started before monsoon to prevent the outbreaks of dengue. This will simultaneously solve the problem of other mosquito borne diseases like malaria, chikungunya, Japanese encephalitis and filaria.

With use of appropriate test; diagnosis of dengue fever can be made early and prompt treatment can be provided to the patient as a result cost of illness due to testing, treatment and duration of hospitalisation can be reduced and valuable time can be saved and number of patient developing complication can be reduced.

Acknowledgments

The authors express their sincere thanks to the principal, J.L.N. Medical College and associated groups of hospital, Ajmer.

References

1. Gupta N, Srivastava S, Jain A, Chautevedi UC. Dengue in India. Indian J Med Res 2012; 136:373-90.
2. Ananthnarayan R, Panikar CKJ, Arboviruses. In Ananthanarayan and Paniker's Textbook of Microbiology, 8th ed. Hyderabad: Orient Longman; 2009: 519
3. Monath TP. Dengue: The risk to developed and developing countries. Proc Natl Acad Sci USA 1994; 91:2395-400.
4. Burke, D.S.,Nisalk, and C.H.Hoke, Jr.1986.Fireld trial of a Japanese encephalitis diagonistic kit.J.Med.Virol.18:41-49.
5. Gubler DJ. Dengue and dengue hemorrhagic fever. Clin Microbiol Rev 1998; 11:480-96.
6. Martina BE, Koraka P, Osterhaus A. Dengue Virus Pathogenesis: an Integrated View. Clin Microbiol Rev. 2009; 22: 564–581.
7. WHO (2002) Dengue and Dengue haemorrhagic fever. Fact sheet No. 117, Geneva.
8. Garg A, Garg J, Rao YK, Upadhyay GC, Sakhuja S. Prevalence of dengue among clinically suspected febrile episodes at a teaching hospital in North India. J Infect Dis Immunity. 2011;3:85–9.
9. Kalawat U, Sharma KK, Reddy SG. Prevalence of dengue and chikungunya fever and their co-infection. Indian J Pathol Microbiol. 2011; 54:844-846.
10. Paramasivan R, Thenmozhi V, Hiriyan J, Dhananjeyan KJ, Tyagi BK, Dash AP. Serological & entomological investigations of an outbreak of dengue fever in certain rural areas of Kanyakumari district, Tamil Nadu. Indian J Med Res. 2006; 123: 697-701.
11. Arya SC, Agarwal N, Parikh SC, Agarwal S. Simultaneous detection of dengue NS-1 antigen, IgM plus IgG and platelet enumeration during an outbreak. Sultan Qaboos Univ Med J 2011;11:470-6
12. Datta S, Wattal C. Dengue NS-1 antigen detection: A useful tool in early diagnosis of dengue virus infection. Indian J MedMicrobio 2010; 28:107-190.
13. Singh MP, Majumdar M, Singh G, Goyal K, Preet K, et al, NS-1 antigen as an early diagnostic marker in dengue: Report from India. Diagon Microbio Infect Dis 2010; 68:50-4.

14. Padbidri VS, Wairagkar NS, Joshi GD,Umarani UB, Risbud AR, Gaikwad DL et al. A serologicl survey of arboviral disease among the human population of the Andaman and Nicobar Islands, India. Southeast Asian J Trop Med Public Health 2002;33;794-800.
15. Baruah J. Incidence of dengue virus infection in a tertiary care centre (Dissertation), Manipal: MAHE 2004.
16. Dash PK, Saxena P, Abhavankar A, Bhargava R and Jana AM. Emergence of dengue virus type 3 in Northern India. Southeast Asian J Trop Med Public Health 2005; 36: 370-77.
17. Neeraja M, Lakshmi V, Teja VD, Umabala P and Subbalakshmi MV. Serodiagnosis of dengue virus infection in patients presenting to a tertiary care hospital. Indian J Med Microbiol 2006; 24: 280-2.
18. Gupta E, Dar L, Kapoor G and Broor S. The changing epidemiology of dengue in Delhi, India. Virol J. 2006;3:92.
19. Kumar A, Rao CR, Pandit V, Shetty S, Bammigatti C, Samarasinghe CM. Clinical Manifestations and Trend of Dengue Cases Admitted in a Tertiary Care Hospital, Udupi District, Karnataka. Indian J Community Med. 2010; 35: 386–390.