



Research Article

Detection of Dengue Virus in Human Blood Samples from Selected Localities of Delhi and NCR

Suresh Chand Kaushik¹, Sukhvir Singh², Purnima Srivastava³

¹Department of Life Science and Applied Sciences, Bhagwant University, Ajmer, Rajasthan, India.

²National Vector Borne Disease Control Programme, Ministry of Health and Family Welfare, 22-Shamnath Marg, Delhi, India.

³Bhagwant University, Ajmer, Rajasthan, India.

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Corresponding Author:

Suresh Chand Kaushik, Department of Life Science and Applied Sciences, Bhagwant University, Ajmer, Rajasthan, India.

E-mail Id:

kaushiksureshchand@gmail.com

Orcid Id:

<https://orcid.org/0000-0002-5141-0232>

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A B S T R A C T

A survey for detection of dengue virus in human blood was conducted. The blood filter paper blots of suspected cases of dengue were collected from different localities of Delhi and NCR. These blood spots were tested using rapid diagnostic kits. In all 87 suspected cases were tested for the presence of dengue virus by repeating them for all confirmation. Out of 87 samples 20 were found dengue positive, 56 samples had past infection and 5 cases were having current fever. The positive samples were again confirmed by PCR and about 8 cases were found positive out of 20 cases. The positivity was found very high from October to November 2016. The Sero conversion rate shows that about 35% of resident are getting new infection each year which is indicator of increasing local transmission. RT-PCR can detect low viraemia which helps in isolating patient and timely control activities. PCR technique along with conventional kits should be carried out to access cases more accurately, so that control can be targeted to such areas.

Keywords: *Aedes aegypti*, RT-PCR, IgM, IgG, NS1

Introduction

Dengue is the most rapidly spreading mosquito-borne viral disease of mankind. It is a major public health concern throughout the tropical and subtropical regions of the world. Almost half of the world's population lived in countries where Dengue is endemic. World Health Organization (WHO) estimates that 50-100 million Dengue infections occur annually with 2200 deaths in more than 100 endemic countries, with a steady increase in the number of countries reporting the disease.¹ Dengue has been identified as one of the 17 neglected tropical diseases by WHO as mentioned in their first report on neglected tropical diseases.² The loss

to the economy is 264 Disability-Adjusted Life Years (DALY_s) per million populations per year.^{3,4} The true numbers are far more, since severe under reporting and misclassification of dengue cases have been documented by the countries.³ Approximately 1.8 billion (more than 70%) of the population at risk for dengue worldwide live in WHO South East Asia Region (SEAR) and Western Pacific Region which bear nearly 75% of the current global disease burden due to dengue.⁵

Dengue virus was isolated in India for the first time in 1945. The first evidence of occurrence of dengue fever in the country was reported in 1956 from Vellore district in Tamil Nadu. The first dengue Demorrhagic Fever (DHF) outbreak



occurred in Calcutta (West Bengal) in 1963.^{6,7} It has been estimated that more than 390 million dengue infections occur every year, of which 96 million manifests clinically and India contributes to about a third global burden of apparent dengue infections.^{8,9}

As per India's Integrated Disease Surveillance Programme, more than 100 dengue outbreaks were reported in 2015.¹⁰ Good laboratory-based disease surveillance is essential for early detection of dengue outbreaks and implementation of effective preventive and control measures. In India, the laboratory capacity for diagnosis of outbreaks under Integrated Disease Surveillance Programme is limited.¹¹ Dengue serotype surveillance is important, as large dengue outbreaks tend to follow changes or re-introduction of serotypes.¹²

The four Dengue Virus Types (DENV1-4), called dengue virus serotypes which differ in nucleotide sequence from each other can co-circulate in the endemic areas because the immunity to one serotype does not afford protection from the infection by a heterotrophic serotype.¹³ Though transmission primarily occurs through bite of a vector, there are reports of dengue transfusion and organ transplantations.¹⁴ Dengue viral infected person may be asymptomatic or symptomatic and clinical manifestations vary from undifferentiated fever to florid hemorrhagic and shock.¹⁵⁻¹⁸

In endemic areas, early symptoms of dengue fever mimic often prevalent diseases such as chikungunya, malaria, viral infections, typhoid, leptospirosis etc. For confirmation of dengue infection, Government of India recommends use of ELISA based antigen detection test (NS1) for diagnosing the cases from the first day onwards and antibody detection test IGM capture ELISA (MAC-ELISA) for diagnosing the cases after the fifth day of onset of disease.¹⁹

There is co-circulation and concurrence of infections by multiple DENV serotypes which has become a frequent occurrence in endemic areas which influence clinical expression and accounts for the emergence of Dengue Hemorrhagic Fevers (DHF).²⁰

DENV serotypes and strains within the serotypes are known to vary in their ability to infect and disseminate in mosquitoes.²¹ Dengue serotype 2 strain have been demonstrated to be better adapted and tend to infect the highly demonstrated *Ae. aegypti* more efficiently.²² It is thus considered the principal vector of DENV in urban areas where the species *Ae. albopictus* is recognized as an important vector in some rural areas.

The Commercial Pathozyme Dengue IgM and IgG (CPD IgM-IgG) test kits are a very good tool for carrying out laboratory-based active surveillance.²³

Molecular diagnosis based on Reverse Transcription

Polymerase Chain Reaction (RT-PCR) has gradually replaced virus isolation methods for the detection of dengue virus in acute phase serum samples. Considering the threat that dengue poses to the country, continuous monitoring of the viruses circulating in different parts of the country would reveal whether there are changes in the virus population from year to year and/or from place to place.

Although several parts of India are endemic for Dengue, Delhi has been worst hit during the past decades. In 1996, DENV-II genotype 4 was circulating in Delhi.²⁴ DENV-II was also reported in 2002 and 2004 while DENV-III in circulation with DENV-II emerged in 2003, replacing DENV-2 in subsequent year.²⁵ In 2006, the co-circulation of DENV-1 with predominance of DENV-III, thereby increasing the risk of severity of the disease in population already exposed to a previous serotype. Such study of epidemiological dynamics in population in which >1 DENV serotype co-circulate simultaneously is of special significance.²⁶

Material and Methods

Delhi and NCR region reporting on an average of 5000-6000 cases every year. The blood filter paper blots (three spots each from one subject) were brought to the laboratory from suspected fever cases from different localities of Delhi & NCR namely Noida, Ghaziabad and Faridabad. The samples were taken randomly twice in a month from the hospitals who had already active fever cases screened through rapid kit IgG, IgM and NS1. Randomly samples were collected from different clinical laboratories during August to December 2016 from Delhi & NCR region to assess the positivity during the study duration.

Blood spots were taken from suspected cases which were already tested using RDT kits. A total of 87 samples were collected for PCR detection. Details of patients were not taken as our objective was to detect fever cases through RT-PCR.

RNA extraction of each bloodspot was done using Qiagen total RNA extraction mini kit (Qiagen, Germany) as per manufacturer instruction. The extracted RNA samples were stored in -70°C.

The samples were subjected to RT-PCR within two days of RNA extraction using Qiagen one step RT-PCR kit (Qiagen Germany). The primers and the nucleotide sequences used in this study were those published by Lanciotti et al. (1992) (Table 1), Reverse transcriptase reaction and amplification was carried out in 50 µL reaction mixture which contained 2 µL of each forward and reverse primer, 8 µL 5X Qiagen one step buffer, 2 µL dNTPs, 1 µL Q one step enzyme mix, and 25 µL RNase free water and 10 µL Sample.

Thermal conditions for PCR were: single step rt reaction was carried out at 50°C for 30 min followed by denaturation 95°C for 15 min, incubation at 94°C for 30 sec for denaturation

of the cDNA, at 55°C for 2 min for primer annealing, and at 72°C for 2 min for primer extension for 40 cycles, followed by incubation at 72°C for 10 min. The amplified products were visualized by electrophoresis on 2% agarose gels with ethidium bromide using TAE (40 mM Tris-acetate, 1mM EDTA) as buffer for the electrophoresis. The bands were visualized under UV light.

travelled Asia for at least one.^{28,29} which show high risk to the international travelers in Asia. The sero-conversion rates are seen very high during the transmission season. However, the conversion rate also depends on the population movements in the megacities like Delhi. High population movement from different areas of India may alter both IgM & IgG. However, new high infection rates (sero-conversion) are indicator of

Table 1. Sequence of dengue primers

S. No.	Primer	Sequence
1.	DEN 1	5'- TCAATATGCTGAAACGCGCGAGAAACCG-3'
2.	DEN 2	5'-TTGCACCAACAGTCAATGTCTTCAGGTTTC-3'

Table 2. Detection of dengue virus from blood samples in Delhi and NCR (August-December 2016)

S. No.	Month	Locality	No. of sample	IgG	IgM	NS1	RT-PCR
1.	August 2016	Nithari Sector-30&Sarfabad (Noida)	9	6	2	0	1
2.	September 2016	Khoda Colony & Railway colony (Ghaziabad)	22	10	4	2	1
3.	October 2016	Uttam Nagar & Najafgarh (Delhi)	28	16	6	1	2
4.	November 2016	Mangolpuri & Raghbir Nagar (Delhi)	18	17	7	2	4
5.	December 2016	Sanjay Colony & Sehatpur Village (Faridabad)	10	7	1	0	0
Total			87	56	20	5	8

The four conventional RT-PCR assays were performed and, in each test, run a volume of 25 µL with 5 µL nucleic acid extract was used as a template.

Result

87 suspected cases were tested for the presence of dengue virus by repeating them for all confirmation. Out of 87 samples, 20 were found dengue positive, 56 samples have past infectious and 5 cases were having current fever. The positive samples were again confirmed by PCR and 8 cases were found positive out of 20 cases. The positivity was found high during October and November 2016 (Table 2). Although sample size in the study area was lower, the concurrent cases in the study area were 4431.

Discussion

Dengue is a viral infection spread mainly through mosquito bite. The infection rate of dengue in our study shows 5.74% which is a high rate. The sero conversion rates was found to be 35% (20 IgM positive/56 IgG positive) which also suggest about 35% of residents are getting new infectious each year. The sample size in this study may be low but it may give trend of dengue infection which shows high transmission during post monsoon season (Aug-Oct). Similar Study by Dhar Chowdhury P et al.²⁷ suggested only 56-97% sero-conversion rates in Dhaka Bangladesh which was very high. In some other study on travels sero-conversion rates were 2.9% in Dutch travelers and 6.7% Israeli travelers who

increasing local transmission or more new cases reporting. Extensive studies conducted on epidemiology of dengue by Vikram et al. (2016) have included high contribution of asymptomatic positive cases which also increases the local transmission risk. There is need to expedite vector control activities with public awareness programs at various levels in such endemic localities to change behaviour for reducing the mosquito genic conditions supporting dengue in urban cities like Delhi. Although rapid kits are reliable but unable to detect low viremia as was wit nested in PCR which detected more than NS1 detection. This prompt a need of better detection method which could also address low viremic patients also in their initial stage which cloud be helpful for isolating patient and timely control activities.

This makes for a public health interest on local DF burden and epidemiology and offers the platform to improve surveillance, laboratory testing capacity and clinical alertness in the health delivery system in Delhi and NCR. Effective prevention and control programs will depend on improved surveillance designed to provide early warning of dengue epidemics. Thus, there is need to study the occurrence of dengue virus infections during the period following an epidemic to provide information on the endemicity of dengue and to predict further epidemics in Delhi and NCR.

Conclusion

It is concluded that the positivity against dengue virus was

found very high from October to November 2016. The Sero conversion rate was found 35% in residents which are getting new infection each year. This indicates that local transmission is going on. PCR technique along with conventional kits should be carried out to access cases more accurately, so that control can be targeted to such areas. Thus, there is need to study the occurrence of dengue virus infections during the period following an epidemic to provide information on the endemicity of dengue.

Conflict of Interest: None

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