

# A comprehensive study of dengue fever patients admitted to a tertiary care hospital in Amritsar

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## ABSTRACT

**Introduction:** Dengue is the most common and widespread arboviral infection in the world today. It is an increasingly prevalent tropical arbovirus infection with significant morbidity and mortality. There are four serotypes of the dengue virus (DENV), i.e., DENV-1, DENV-2, DENV-3, and DENV-4 circulating in Indian population and can cause the full spectrum of disease from a subclinical infection to a mild self-limiting disease, the dengue fever (DF), severe disease that may be fatal, and the dengue hemorrhagic fever/dengue shock syndrome. The present study was done to do comprehensive study of DENV infection in Amritsar. **Materials and Methods:** Blood samples from clinically suspected patients of DF selected as per the WHO criteria. Samples were screened for the presence of dengue-specific immunoglobulin M antibodies using M antibody capture-enzyme-linked immunosorbent assay (ELISA) and dengue non-structural protein 1 (NS1) antigen detection was done using Pan Bio (Australia) NS1 ELISA kit, according to the duration of fever as per National Vector Borne Disease Control Program guidelines. **Results:** A total of 3306 samples were tested for dengue. 89.7% ( $n = 2966$ ) samples were positive for both seromarkers of dengue. Seasonal variation of disease was seen and majority of cases tested positive for dengue were obtained in September and October. Male predominance was seen and the affected male:female ratio of patients suffering from dengue in this study was found to be 1.8:1. Adult age group had higher burden of disease than the pediatric age group and majority of patients suffering from dengue came from urban background. **Conclusion:** This study helps in understanding the burden of infection. Early and definite diagnosis is necessary to lower the morbidity and mortality. Continued and coordinated effort should be made to prevent dengue outbreaks.

**Key words:** Dengue, immunoglobulin M antibody capture enzyme-linked immunosorbent assay, non-structural protein 1 antigen

## INTRODUCTION

Dengue is the most common and widespread arboviral infection in the world today. It is an increasingly prevalent tropical arbovirus infection with significant morbidity and mortality.<sup>[1]</sup> Dengue infection has been known to be endemic in India for over 2 centuries.<sup>[2]</sup> It represents substantial disease burden in many tropical and sub-tropical countries, particularly in children and young adults, predominantly in urban and semi-urban areas.<sup>[3]</sup> With more than one-third (2.5 billion) of the world's population living in areas at risk for transmission, dengue is considered as the leading cause of illness and death in the tropics and subtropics. As many as 50 million infections occur worldwide and this number is projected to increase.<sup>[4]</sup>

Dengue viruses (DVs) belong to the family Flaviviridae, and there are four serotypes of the virus referred to as DENV-1, DENV-2, DENV-3, and DENV-4.<sup>[1]</sup> DV is a positive-stranded encapsulated RNA virus and is composed of three structural protein genes, which encode the nucleocapsid or core (C) protein, a membrane-associated (M) protein, an enveloped (E) glycoprotein, and seven non-structural (NS) proteins. It is transmitted mainly by *Aedes aegypti* mosquito and also by *Ae. albopictus*. All four serotypes, i.e., DENV-1 to DENV-4 can cause the full spectrum of disease ranging from a mild self-limiting disease, the dengue fever (DF), to life-threatening dengue hemorrhagic fever and dengue shock syndrome.<sup>[1]</sup>

The purpose of this study was to do comprehensive study on the diagnosis of DF cases admitted to our tertiary care hospital from January 2016 to December 2016.

## MATERIALS AND METHODS

The study was done in the Microbiology Department of Government Medical College, Amritsar. Clinically suspected patients of DF along with the presence of headache, myalgia, retro-orbital pain, rash, and hemorrhagic manifestations selected as per the WHO criteria<sup>[4]</sup> presenting to the emergency, outpatient and indoor services of our institute were included in our study from January 2016 to December 2016. As this institute provides specialty health care to neighboring districts, blood samples were also received from neighboring districts. Demographic details and clinical history were obtained and recorded in laboratory request forms.

About 5 ml of blood sample was collected aseptically by venipuncture in a plain vacutainer from clinically suspected DF patients and allowed to clot at room temperature and then centrifuged at 2000 rpm for 10 min. The sera separated after centrifugation was aliquoted into sterile 1.5 ml storage vials. As per National Vector Borne Disease Control Program, if duration of fever in patients was more than 5 days, the samples were

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**Table 1: Percentage positivities of NS1 antigen and IgM antibodies during 2016**

Serological tests	Positive cases (%)
NS1 antigen	1254 (42)
IgM antibody	1712 (58)
Total positives	2966

NS1: Non-structural protein 1, IgM: Immunoglobulin M

**Table 2: Characteristics of patients with dengue fever**

Symptoms	Number of cases (%)
Fever	2847 (96)
Myalgia	266 (9)
Headache	237 (8)
Arthralgia	177 (6)
Vomiting	88 (3)
Skin rash	59 (2)
Retro-orbital pain	16 (0.5)
Hemorrhagic manifestations	6 (0.2)

DF: Dengue fever

screened for the presence of dengue-specific immunoglobulin M (IgM) antibodies by IgM antibody capture enzyme-linked immunosorbent assay-ELISA) using kit prepared by the National Institute of Virology, Pune, India, strictly following the manufacturer's protocol.

In case of, acute DF cases (fever duration  $\leq 5$  days) detection of dengue NS protein 1 (NS1) antigen in the acute sera was done using Pan Bio (Australia) NS1 ELISA kit.

## RESULTS

During the study period, a total of 3306 samples were tested for dengue. Of total sample tested, 89.7% ( $n = 2966$ ) were positive for both seromarkers of dengue Table 1. Majority of cases tested for dengue were obtained in September ( $n = 1346$ ) and October ( $n = 1278$ ). A gradual increase in dengue positive cases was noticed from August ( $n = 104$ ) with a highest peak in September ( $n = 1293$ ) and October ( $n = 1170$ ) followed by decline in positive cases in November ( $n = 377$ ). Minimal cases were seen in June and July ( $n = 1$  and  $n = 2$  respectively). No case was found to be positive from January to May [Figure 1].

On analyzing data, male predominance was seen in samples tested for DF. Of the total samples tested for DF, 2104 samples were of male patients and 1202 were of female patients. Percentage positivities in male and female patients suffering from DF were 64% ( $n = 1898$ ) and 36% ( $n = 1067$ ) out of total positive cases for dengue [Figure 2]. Therefore, the affected male:female ratio of patients suffering from dengue in this study was found to be 1.8:1. Maximum number of patients tested for DF were found in adult age group, i.e., 18–35 years followed by patients of 5–17 years of age. Adult age group showed 56% ( $n = 1667$ ) seropositivity for dengue while pediatric age group showed 19% ( $n = 567$ ) seropositivity for dengue out of total positive cases [Figure 3].

Majority of samples received for testing mainly belonged to patients living in urban areas. Dengue serology was positive in

59% cases ( $n = 1749$ ) in samples from urban areas and 41% positive ( $n = 1216$ ) in samples coming from rural areas out of total laboratory-confirmed cases of DF.

Among clinical features of dengue, fever was present in almost all the patients, i.e., 96% patients ( $n = 2847$ ). Myalgias were seen in 266 cases (9%), headache in 237 (8%), and arthralgias in 177 (6%) cases. Vomiting (3%), skin rash (2%), and retro-orbital pain (0.5%) were found in lower proportions among patients with DF. Hemorrhagic manifestations were found in 6 cases only Table 2.

## DISCUSSION

To prevent the dengue outbreaks, it is necessary to diagnose the DENV infection as early as possible.<sup>[5]</sup> In this study, 89.7% patients were infected with DENV out of total samples tested. NS1 antigen positive percentage was 42% and dengue-specific IgM antibody percentage was 58% out of total positive samples, indicating increased DENV activity.

On analysis of data on monthly basis, seasonal variation of disease was seen. The seasonality of transmission of dengue with increased activity in monsoon and post-monsoon season was seen in the present study which was in accordance with the reported patterns of dengue transmission in other studies.<sup>[2]</sup> The infection started spreading in August, peaked during September and October and started to taper during November. This can be explained by stagnant water sources following heavy rainfall favoring breeding of the mosquito vector resulting in increased post-monsoon incidence of dengue, thereby maintaining the vector population throughout the year.<sup>[6]</sup>

Effective control measures and preventive measures should come into full swing during water stagnation periods after the initial bouts of rainfall and at the end of monsoon.<sup>[7]</sup> The higher prevalence of dengue infection was noted among males than females. The male to female ratio was 1.8:1 which correlates well with other studies undertaken in North India<sup>[2,8]</sup> and South India<sup>[9]</sup> where male affliction of the disease has been reported. High prevalence among males is probably due to more outdoor activities by males in comparison to females which results in more exposure today biting mosquitoes.<sup>[7]</sup> The higher male disease could also be attributed to gender bias in seeking health care because males have preference over females in traditional societies in Asian countries.<sup>[10]</sup> In this study, dengue positivity was high in patients of age group 18–35 years of age followed by 5–17 years age group. These findings are consistent with other Indian studies.<sup>[9,2,11]</sup> Antony *et al.*,<sup>[12]</sup> Kumar *et al.*, and<sup>[9]</sup> Mehta *et al.*<sup>[13]</sup> found common age group was above 30 years. Another study in Kanpur, showed 0–15 years group to be commonly affected.<sup>[14]</sup>

The current study shows fever was invariably present in all the laboratory confirmed cases. Similar studies in and around India have also substantiated fever as being the most common presenting symptom. Retro-orbital pain, generally considered as a cardinal feature of DF, was scarcely seen.<sup>[9]</sup> Another study from Delhi saw epistaxis and melena to be the most common manifestation, whereas a study from South India reported rash and petechiae to be the common ones.<sup>[9,15]</sup> Majority of patients

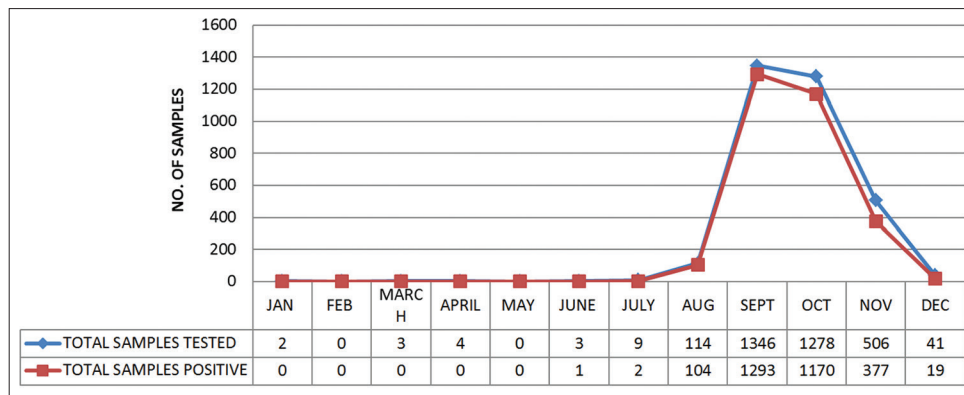


Figure 1: Month wise distribution of dengue cases in 2016

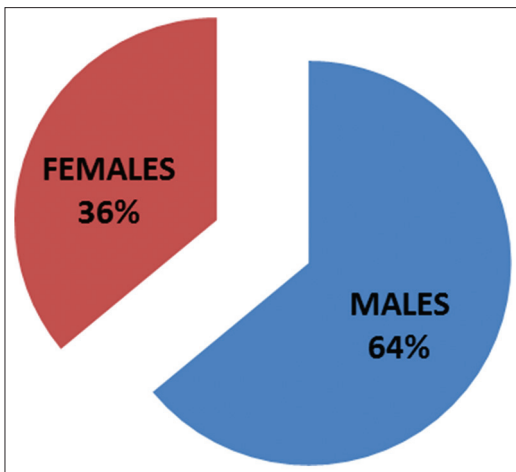


Figure 2: Sex distribution among positive cases

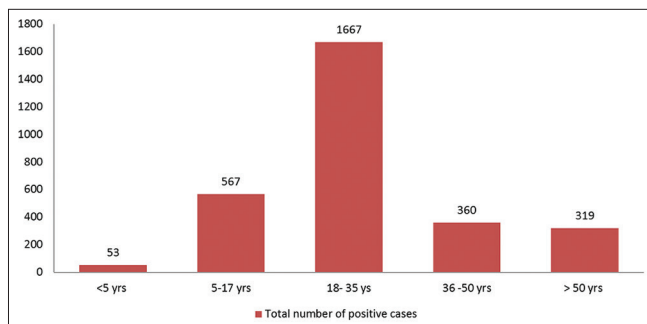


Figure 3: Age wise distribution of dengue cases in 2016

belonged to urban areas of the region, who were confirmed dengue positive. High burden of dengue in urban areas can be owing to rapid unplanned urbanization with unchecked construction activities that contribute to fertile breeding grounds for mosquitoes.

### CONCLUSION

Dengue has become a major public health problem in India. It has become endemic in this region of North India as a large number of cases are being reported every year. In our study, seasonal trend of disease was seen and most cases are seen

during the post-monsoon period indicating that preventive measures against dengue infection should be taken during post-monsoon water stagnation periods. Early and definite diagnosis is necessary to lower the morbidity and mortality. Continued and coordinated effort should be made to prevent dengue outbreaks. This study emphasizes the need for continuous seroepidemiological surveillance for the timely formulation and implementation of effective dengue control program.

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