



# TO STUDY THE PREVALENCE OF DENGUE ITS SEROTYPES WITH SPECIAL REFERENCE TO THE CYTOKINE PROFILE IN PRIMARY AND SECONDARY CASES OF PATIENTS

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## ABSTRACT

**Introduction :** Dengue virus is a positive stranded RNA virus belonging to genus *Flavivirus* and family *Flaviviridae*. Dengue virus can infect infants, young children and adults. Dengue fever is an emerging arboviral public health problem in a large endemic population in the tropical and sub-tropical areas of the world, with varying degrees of clinical presentation.

**Aim and Objective:** This study characterizes dengue cases and to find out the clinical profile along with the cytokines.

**Material and methods:** This was a cross-sectional study carried out in the Department of Microbiology at a tertiary care centre for a period of 12 months i.e, January 2023 to December 2023. Prevalence was estimated by analyzing data from the results of IgM antibody by ELISA tests and variations in disease reporting by gender, age, serotypes, clinical manifestation and season were assessed.



**Results:** In the present study a total of 450 samples were analyzed during the study period. Out of which 359 samples were found positive for dengue infection. Age group of 11–20 years was found to be the most infected by Dengue in which males are more commonly infected than females. Of those 4 Serotypes in overall samples, 39.55 % were caused by DENV-2, 30.64 % by DENV-3, 21.44 % by DENV-1, and 8.35% by DENV-4. The prevalence of Dengue was from August to November might be due to the rainy season. Clinical manifestation fever was observed to be 100%, headache 43.17%, and abdominal pain 44.01% was found in most of the dengue confirmed positive patient while altered sensorium 19.77% and positive tourniquet test 15.32% least found [in patients](#). **Conclusion:** The present study showed that dengue infection was more prevalent among young males. Awareness and educating the community regarding mosquito breeding habits, prevention, and control of the vector will help in controlling the dengue. Development and use of an effective vaccine will be beneficial in endemic areas to help and limit the spread of infection.

**Keywords:** Serotypes, DEN, IgM, ELISA, cytokines

**DOI Number:** 10.48047/nq.2024.22.5.nq25011

**NeuroQuantology 2024; 22(5):114-126**

## INTRODUCTION

Dengue is a mosquito-borne arboviral illness that poses a significant global public health issue in the tropical and subtropical parts of the world, particularly in urban and semi-urban areas [1]. Dengue is a mosquito-borne arboviral illness that poses a significant global public health issue in the tropical and subtropical parts of the world, particularly in urban and semi-urban areas [1].

Globally, it is estimated that 3.9 billion people from more than 128 countries are at risk of dengue virus (DENV) infection, with 284–528 million cases occurring each year, of which 96 million manifest clinically with a severe form of the disease [2,3]/

The World Health Organization (WHO) estimates that approximately 50-100 million individuals are infected with dengue annually, and more than 2.5 billion individuals live at risk in more than 100 countries of dengue transmission. Rapid unplanned urbanization and migration of population from rural to urban areas, lack of vector control, climatic changes, and poor sanitation facilities have contributed to fertile breeding areas for the dengue vector, *Aedes aegypti* [2].

DENV is a positive-sense single-stranded RNA whose genome is 11 kb in length which encodes

three structural (capsid, membrane, envelope) and seven non-structural (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) proteins [7].

Dengue is caused by four different serotypes of the dengue virus (called DENV 1, DENV 2, DENV 3, and DENV 4), and infection can occur by any one or more than one of the four serotypes. Infection with any one dengue serotype provides lifelong homotypic immunity to that particular serotype. It is well-documented that subsequent infections with different dengue virus serotypes increase the risk of developing severe dengue [3].

The clinical manifestations of dengue range from self-resolving dengue fever (DF) to the more severe dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). Immunity to one serotype does not protect against other serotypes (4). DHF, the more severe type, is primarily seen in people who develop a heterotypic secondary infection [5]. Dengue's severity varies with infected persons' age [6] [7], the infectious DENV serotype/genotype [8] [9], immunological status [10], and population genetic makeup [11]. Humoral immunity was thought to be the most important contributing element, as evidenced by in vitro antibody dependent enhancement (ADE) [12] and sero-epidemiological studies [13], [14], [15].

Later, the infection causes a systemic viral infection, and complications are associated with the pathogenic role of endothelial cells [16-18]. Cytokines increase vascular permeability and hemorrhage during



dengue infection. These molecules are proteinaceous and are secreted during innate and adaptive immunological responses, acting as inflammatory mediators or modulatory molecules during dengue infection [19,20]. Viral infection triggers the innate immune response, which produces pro-inflammatory cytokines that recruit and activate cells involved in inflammation and the formation of adaptive immunity. Cytokines also assist discriminate between T-helper 1 (Th1) and T-helper 2 (Th2) cells, and their role in dengue has been extensively studied [21]. The dengue virus is tropic and replicates in human monocytes, macrophages, and hepatocytes, all of which produce cytokines [22].

Numerous studies have demonstrated that the concentrations of cytokines, mediators and soluble receptors are significantly increased during DENV infection, this dysregulation of certain cytokines has major implications in dengue pathogenesis especially in DHF and DSS [21,22]. By now, it is common knowledge that DENV can infect various immune cells like dendritic cells and monocytes which contribute to the production of inflammatory cytokines. This increased unregulated production of cytokines can aggravate pathogenesis, organ failure and cause death [23].

Dengue fever is a severe sickness similar to the flu that has a 4-10 day incubation period. Typically, symptoms persist 2-7 days. Dengue is divided into two major groups by WHO: dengue (with or without warning signals) and severe dengue [23]. Dengue fever was formerly only seen in cities, but it is increasingly spreading to rural areas. Many reasons contribute to the expansion of Dengue infection in India, including changes in environmental conditions, unplanned urbanisation, host-pathogen interactions, and some immune characteristics of the population. Inadequate vector control measures have also created favorable conditions for dengue virus transmission and its mosquito vectors [24][HYPERLINK "https://www.sciencedirect.com/science/article/pii/S2772431X23000035"](https://www.sciencedirect.com/science/article/pii/S2772431X23000035)

[e/pii/S2772431X23000035](https://www.sciencedirect.com/science/article/pii/S2772431X23000035)]. Isolation of virus by cell culture and molecular detection<sup>4</sup> techniques not only require costly laboratory setups but these techniques are time consuming and mostly available in research or reference laboratories. Detection of NS1 antigen and IgM/[IgG antibodies](https://www.sciencedirect.com/science/article/pii/S2772431X23000035) specific to Dengue virus by ELISA remains an important diagnostic tool in resource limited settings [\[2 HYPERLINK "https://www.sciencedirect.com/science/article/pii/S2772431X23000035"5 HYPERLINK "https://www.sciencedirect.com/science/article/pii/S2772431X23000035"\]](https://www.sciencedirect.com/science/article/pii/S2772431X23000035).

Molecular approaches, such as reverse transcription-polymerase chain reaction (RT-PCR), can diagnose DENV on the same day during the acute phase of the disease (phase with viremia) and determine the serotype. PCR-based techniques outperform virus isolation methods in terms of sensitivity, specificity, speed, simplicity, and cost [26].

The present study was therefore undertaken to estimate the prevalence of dengue, its serotypes with special reference to the cytokine profile in primary and secondary cases of patients attending a tertiary care centre and to analyze the trend of the dengue infection.

#### **MATERIAL AND METHODS**

The present study was a Cross-sectional study conducted in the Department of Microbiology at a tertiary care centre for a period of 12 months i.e, January 2023 to December 2023.

#### **Inclusion criteria:**

Patients with dengue like illness were included

#### **Exclusion criteria:**

Those with parasitic and respiratory infections were excluded by the clinicians.

#### **Sample Processing**

Blood samples were transported on ice, plasma was separated and aliquoted. Out of the 450 suspected dengue patients, 359 attended the department, and were hospitalized. The individuals were tested for



the presence of DENV-specific IgM and/or viral RNA and those positive by either of the two tests were considered as dengue patients. The clinical presentations of the patients were recorded by the clinicians of the respective hospitals, and abstracted by chart review. Patients with fever, headache, myalgia, retro-orbital pain, and rash were defined as DF. DHF patients were categorized by the presence of at least two of the DHF defining criteria of the World Health Organization: hemorrhagic tendencies/manifestations, thrombocytopenia, and evidence of plasma leakage.

### Sample collection and Laboratory test

Blood samples of patients presenting as probable dengue fever having fever of more than 5 days duration were collected from day 1–7 days of illness. A volume of 3–5 mL of blood was collected in plain serum vial under aseptic conditions and transported to the laboratory within 6 hours of collection maintaining cold chain at 4 °C. Serum was separated using standard methods and subjected to DEN-specific IgM and [IgG antibodies](#) by dengue IgM capture ELISA test kit supplied by NIV, Pune. The test procedure was performed as per instructions given by the manufacturer. As per manufacturer's guidelines values calculated and results were interpreted. A standard sample requisition form was collected containing clinical and epidemiological information of the patients.

### Cytokine measurement

Blood specimens were collected for cytokine analysis during the three phases of infection. A commercial assay (Bio-Plex Human Cytokine Assay; Bio-Rad Inc., Hercules CA) was performed to detect the levels of IL-2, IL-4, IL-6, IL-8, IL-10, interferon gamma (IFN $\gamma$ ), and TNF $\alpha$ . In brief,

sera obtained from participants during the three phases were mixed with beads coated with antibodies to cytokines and a unique fluorescent intensity. Subsequently, the mixtures were incubated with biotinylated anti-cytokine antibodies.

Finally, phycoerythrin-conjugated streptavidin was added, and the fluorescent signals were detected using a multiplex array reader (Bio-Plex 200 System, Bio-Rad Inc., Hercules, CA). Raw data were initially measured as the relative fluorescence intensity and then converted to cytokine concentration based on a standard curve generated from the reference concentrations.

### Statistical analysis

Data was analysed using WHO Epi info software version 3.5.4. Observations were presented as frequency and percentage distribution. Relevant descriptive statistics like frequency and percentage were calculated for presentation of data.

### Ethical clearance

The ethical committee clearance certificate was taken before starting of study by Institutional Medical Ethical Committee.

### RESULTS

In the present study, among 450 clinically suspected cases, 359 were found to be positive for dengue infection. Of those 359 total positive cases of dengue, the proportion of cases was maximum in the age group of 31-40 being affected the most followed by 21-30 and least was observed in the age group above 60 years of age as shown in Table No. 1. and Graph No.1 shows gender wise distribution of 359 total positive cases of dengue in which larger representation of males 205 (57.10%) as compared to females 154 (42.89%) respectively.

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Age(inyears)	No.ofcases	Percentage
0-10	74	20.61%
11-20	108	30.08%
21-30	79	22.0%
31-40	37	10.30%
41-50	23	6.40%
51-60	21	5.84%
>60	17	4.73%

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**Table No 1: Age wise distribution of positive cases.**

**Graph No. 1: Gender wise distribution of positive cases.**

In the current study, about 37.32% (134 patients) had primary dengue infection and 62.67% (225 patients) had primary dengue infection as shown in Graph no. 2.

**Graph No. 2 : Distribution based on primary and secondary dengue.**

Table no. 2 shows the clinical manifestation of patients such as fever, headache, abdominal pain and nausea/vomiting, retro-orbital pain, [petechiae](#), altered sensorium, positive tourniquet test were



reported. Among the clinical manifestation fever 100%, headache 43.17%, and abdominal pain 44.01% was found in most of the dengue confirmed positive patient while altered sensorium 19.77% and positive tourniquet test 15.32% least found [in patients](#) .

ClinicalManifestation	No.ofcases	percentage
Fever	359	100%
Headache	155	43.17%
Abdominalpain	158	44.01%
Nausea/vomiting	138	38.44%
Retro orbitalpain	130	36.21%
Petechiae	99	27.57%
alteredsensorium	71	19.77%
Positivetourniquetest	55	15.32%

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**Table No. 2: Clinical manifestation of dengue positive patients.**



In the present study, of those four Serotypes in overall samples, 39.55 % were caused by DENV- 2, 30.64 % by DENV-3, 21.44 % by DENV-1, and 8.35% by DENV-4. Therefore, DENV-2 was predominant (39.55%) in our study as shown in Table no.3

Serotypes	No. of cases	Percentage
DENV-1	77	21.44%
DENV-2	142	39.55%
DENV-3	110	30.64%
DENV-4	30	8.35%

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**Table No. 3: Distribution of patients on the basis of serotypes.**

In our study, a total 359 positive cases of dengue where 54.31% (n= 195) of the patients were classified as DF, Presence of any two clinical features of the DHF defining criteria by WHO, categorized 164(45.68%) patients as DHF cases as shown in Table no.4.

<b>Diseasecategories</b>		
<b>DF</b>	<b>195</b>	<b>54.31%</b>
<b>DHF</b>	<b>164</b>	<b>45.68%</b>

**Table No. 4: Distribution on the basis of clinical features.**

In the current study, the circulating levels of four cytokines, IFN- $\gamma$ , TNF- $\alpha$ , IL-6 and IL-8 were assessed in the 359 confirmed dengue positive cases as shown in Graph no.3.

**Graph No.3: No. of cases with increased values of cytokines in DF/DHF.**

The number of cases showing increased IFN- $\gamma$  levels was larger in the DHF category 26.80% as compared to DF cases 33.84%. The value of TNF-  $\alpha$  and IL-6 was almost equal in both DF and DHF. However, with disease categorization, the levels of IL-8 in DHF 46.34% were higher than in DF 38.97% patients.

Graph No. 4 shows the prevalence of dengue positivity was from August to November might be due to the rainy season. Furthermore, some dengue positive cases were also reported in January and March season.

**Graph No. 4: Month wise distribution of dengue cases.**





## DISCUSSION

Dengue is an arboviral disease and it is endemic throughout the world including India. Dengue is caused by four different [serotypes](#): DENV-1, DENV-2, DENV-3, and DENV-4. Laboratory confirmation of dengue virus infection involves detection of the virus, viral [nucleic acid](#), antigens or antibodies, or a combination of these techniques. In India, National [vector borne disease](#) control program (NVBDCP) and state government perform dengue surveillance *via* testing the suspected patients samples. WHO has declared dengue as hyper endemic in India and cyclical epidemics of dengue are becoming more frequent. There has been a steady rise in the number of dengue cases reported every year.

In our present study, 450 patients clinically suspected of dengue fever was tested for the dengue viral disease out of which 359 (79.7%) were found to be positive for dengue infection. There was another research investigator Singh S and Jahan S in 2021 stated that Out of the 314 cases tested, 117 cases (37.2%) were tested positive for one or more of three markers [26]. The present study was in support to the study conducted by the other author Mishra S in 2019 where the total positive cases studied were 350 [27].

In the present study, the prevalence of serologically positive for dengue virus out of total 359 samples tested was 79.7%. This seropositivity of dengue cases was correlated with Nidhi Pandey et al [28] and Singh S [26] 37.2 % but was in contrasts with the study by K.P. Modi et al. [29].

The present study observed maximum positive cases 359 was observed in the age group of 11-20 years with 108(30%) followed by 21-30 years with 79 (22%), 31-40 years with 37 (10.3%). This study was in support with the another study where the maximum number of cases recorded were in <sup>11</sup> the age group of 26-45 years of age followed by 18-25 years of age and least in the age group of 60 years and above [27]. Similar to findings of several study by Gupta et al [30] and Chakravarti [31] and Kumari A [32] where high numbers of samples were from 21–

30 years and 31-40 age while less number of samples from older age group such as 61– 70 years and 71–80 years received. Study by Singh S [26] also recorded the similar finding where Adults between the ages 21-40 years were found to be the most vulnerable group as 104 (53%).

In the present study, the higher prevalence of dengue infection was found more in males 205 (57.10%) as compared to females 154 (42.89%) respectively.

This finding was in accordance to study conducted by D. Priyadarshini [33] and Garg A [34] where males are more commonly affected than females. Another study was also found in accordance to the current study where the males to be more as compared to the females, as well as it was observed that almost all the patients were between the age of 18-60 years thereby indicating that it may be because of the occupational exposure to the vector of dengue infection [27].

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In the current study, the clinical manifestation fever was observed to be 100%, headache 43.17%, and abdominal pain 44.01% was found in most of the dengue confirmed positive patient while altered sensorium 19.77% and positive tourniquet test 15.32% least found [in patients](#) which was similar to other study by Mohamed [35], D. Priyadarshini [33] Mishra [27] and kumar A [32]. Singh S also recorded the most common symptoms to be fever (100%), myalgia/ arthralgia (71.1%), headache (48.88%), Rash (27.77%) [26].

The number of cases showing increased IFN- $\gamma$  levels was larger in the DHF category 26.80% as compared to DF cases 33.84%. The value of TNF-  $\alpha$  and IL-6 was almost equal in both DF and DHF. However, with disease categorization, the levels of IL-8 in DHF 46.34% were higher than in DF 38.97% patients.

In our study, a total 359 positive cases of dengue where 54.31% (n=195) of the patients were classified as DF, Presence of any two clinical features of the DHF defining criteria by WHO, categorized 164(45.68%) patients as DHF cases.



Similar study also show the same conclusion conducted by Innis BL [36] , Vaughn DW [37] and Chanama S[38] .Thus in early phase of dengue NS1 and PCR are better tests for diagnosis and later IgM is better. This is true for both primary and secondary dengue fever.

A seasonal trend of dengue over one year period was assessed. In the present study common symptoms observed in dengue-positive dengue cases were found to be maximum in August to September during monsoon season. Similar pattern is reported by DeshkarST[39], Kumar A[32] and Singh J[40].The reason for monsoon peak in our study may be due to prime occupation of the people being agriculture and increased breeding of vector mosquitoes owing to collection of rain water in domestic and peri domestic areas.

In the present study the number of cases showing increased IFN- $\gamma$  levels was larger in the DHF category 26.80% as compared to DF cases 33.84%. The value of TNF-  $\alpha$  and IL- 6 was almost equal in both DF and DHF. However, with disease categorization, the levels of IL-8 in DHF 46.34% were higher than in DF 38.97% patients. This study was in accordance to the other study by Sri Masenyi,et al. in 2024 where Cytokine levels (TNF- $\alpha$ , IL-6, IL-10, and IL-17) were measured using enzyme-linked immunosorbent assay (ELISA). Infection status was determined by combining serological and RT-PCR results, categorizing patients into primary and secondary infections. It was observed that DF patients had lower TNF- $\alpha$ , IL-6, and IL-17 but higher IL-10 levels compared to DHF patient ( $p < 0.001$ ). Elevated TNF- $\alpha$ , IL-6, and IL-17 levels were higher in secondary infection, while IL-10 level was higher in primary infection [41].

Cytokines play a crucial role in the interplay between cytokine dysregulation and dengue infection dynamics. DHF cases among patients with primary infections have been documented and even in primary cases, the progress from DF to DHF has been associated with increased levels of tumour necrosis factor (TNF)- $\alpha$ , interleukin (IL-1), and IL-6 .Thus, abundant evidence suggests that high cytokine levels have a role in DHF development [42]. Regular surveillance to detect and monitor sudden

increases in the numbers of dengue cases are important [43,44].

## CONCLUSIONS

The current study found that dengue infection was more common among young males. Fever, headache, and stomach pain were among the few

patients.

Increasing community awareness and education about mosquito breeding behaviours, vector avoidance, and control will aid in dengue control. The development and administration of an effective vaccine will benefit endemic areas by assisting in the prevention and control of infection transmission. The understanding and interaction between the many serotypes and clinical symptoms can aid in determining the various factors contributing to illness severity.

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