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Research Article

Serotype distribution and association of dengue viral load with clinical severity: A serotype-specific qRT-PCR study

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Abstract

Background: Dengue fever is a viral infection caused by arbovirus; an enveloped single standard RNA virus belongs to the family Flaviviridae. Since last two years, onward from 2023, changes in climate, urbanization and distribution pattern of biological vectors leads to increase transmission of dengue virus. In recent outbreak, all four serotype of dengue virus DENV-1, DENV-2, DENV-3 and DENV-4 have been detected in Pakistan.

Methods: The current prospective cross-sectional study was conducted from March 2025 to October 2025 at Allama Iqbal Teaching Hospital Dera Ghazi khan. A total of 278 dengue suspect patients were enrolled in the study and were using serotype specific quantitative real-time polymerase chain reaction (qRT-PCR).

Results: The mean age of the participant was 35 years ranging from 15 to 76 years and there was a male predominance (64.4%). The results of current study showed that among these 278 tested samples, 28 were positive for dengue virus as confirmed by qRT-PCR. Among the 28 confirmed dengue cases, DENV-2 was most prevalent serotype 16/28, followed by DENV-1 8/28, DENV-3 3/28 and DENV-4 was found to be least prevalent (1/28). Seasonal distribution analysis showed highest positivity in the months of August and September with 7 and 10 positive cases respectively. The results of present study also showed that the mean Ct value for DENV-1, DENV-2, DENV-3 and DENV-4 were 27.8 ± 4.6, 23.9 ±3.5, 28.1 ±5.0 and 28.4 ±4.4 respectively. It was observed that individuals infected with serotype DENV-2 had significantly lower Ct value which indicated higher viral load. Patients with higher viral load showed severe signs and symptoms as well.

Conclusion: DENV-2 serotype was found to be the most prevalent serotype during 2025 dengue outbreak followed by DENV-1, DENV-3 and DENV-4 in studied region. Higher viral load was directly proportional to severity of disease particularly in patient infected with DENV-2.

Key Words: Dengue virus, Serotype, DENV-2, qRT-PCR.

INTRODUCTION

Dengue fever is a viral infection caused by arbovirus; an envelope single standard RNA virus belongs to the genus flavivirus family Flaviviridae. As dengue fever is spreading rapidly all around the globe, it is emerging as one of the major health concerns for public and health workers. It has been noted that dengue virus affects more than hundred million people globally per year. It causes over 20,000 deaths yearly and mainly infects children [1]. Since last two years, onward from 2023, distribution pattern of biological vectors has led to the increased transmission of dengue virus. It is estimated that almost 5 million cases with more than 5000 dengue related death were recorded in countries like Pakistan, Afghanistan, Somalia and Yemen [2].

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Aedes mosquitoes serve as biological vectors for dengue virus and spread the virus by biting the people after contact with infected individuals. Aedes Egypt and aedes albopictus are two main species of mosquito to carry dengue infection [3]. Climate change influences the incidence rate of dengue fever because the lifecycle of this vector depends on availability of fresh water (rainfall) and ambient temperature. It has been observed that dengue outbreak is directly associated with rainfall, moderate temperature and relative humidity [4]. So far, four serotypes of this virus have been identified i.e DENV-1, DENV-2, DENV-3 and DENV-4. Infection with every single serotype develops lifelong immunity against that specific serotype but not against the other ones. This can lead to occurrence of severe secondary infection of dengue virus due to pathological phenomena in which pre-existing antibody help the pathogen to get enter to the host cell and this is called as antibody dependent enhancement [5].

In Pakistan, researchers reveal that in early outbreak of dengue in nineties, DENV-2 and DENV-3 serotypes were more prevalent. However, the prevalence of serotype changed with time. In recent outbreaks in Lahore and peripheries, DENV-1 and DENV-2 serotypes were more common [6]. With advance laboratory testing like polymerase chain reaction, the serotyping dengue virus from an infected subject can be performed on very early stage of dengue fever [7]. Dengue fever shows mild to severe clinical manifestation in different patients. It has been observed that secondary dengue infection shows more severe signs and symptoms as compared to primary infection. Individual with compromised immune system, pregnant women, diabetic patient or a person dealing with other chronic disease may present more clinical presentation [8]. In recent decades, dengue hemorrhagic fever and dengue shock syndrome have been recorded in Pakistan. At early stages, dengue hemorrhagic fever shows variety of specific and non-specific symptoms like fever, severe headache, discomfort, muscle pain, petechiae and rashes. In critical stages, plasma leakage occurs that leads to shock and severe bleeding. Patients with illness may get renal failure, liver dysfunction, gastrointestinal bleeding and encephalopathy [9]. Until now, there is no specific treatment available for dengue viruses, however low-grade fever and body pain can be treated with antipyretic and painkiller, but severe cases must be admitted in hospital and fluid therapy must be initiated [10].

METHODS

Study Design and Setting

After ethical approval by ethical review committee of Allama Iqbal Teaching Hospital Dera Ghazi Khan under letter number AITH-209-25, the current prospective cross-sectional study was conducted in Central pathology laboratory and Biosafety level III laboratory from March 2025 to October 2025.

Patient Cohort

A total of 278 dengue suspect patients were enrolled in the study. After taking consent from the patients and their attendants in case of children, the serum sample were processed for polymerase chain reaction. The mean age of the participant was 35 years ranging from 15-76 years and there was a male predominance (179/278=64.4%). Medical history and clinical manifestation of the patients were recorded on a standardized questionnaire designed by the laboratory official for routine working.

Sample Processing

The samples were processed for quantitative real-time polymerase chain reaction (qRT-PCR) for detection and identification of dengue virus serotypes. Samples were taken from the patients by venipuncture technique under aseptic condition and were inserted into serum separator tube and all the specimens were centrifuged at 3000rpm for 5 minutes to obtain serum. For the extraction of viral RNA, Bosphore Viral RNA spin Kit was used. 150ul of patient serum was added in RNA extraction kit and proceed as per manufacturer guidelines. TANbead auto extraction was used for the extraction of dengue viral RNA under control conditions. TANbead auto extractor uses magnetic beads for RNA extraction and consists of series of steps mainly includes lysis, binding, washing and elution to purify and isolate RNA of given sample.

Real Time PCR

After extraction, the isolated RNAs were further preceded for quantitative real-time PCR. Anatolia Geneworks PCR Thermal Cycler machine was used to perform PCR. Master mix containing essential ingredients like reverse transcriptase that convert viral RNA into complimentary DNA, serotype specific primers of each type DENV-1 to DENV-4, taq polymerase enzyme that adds nucleotides to make daughter DNA strands and buffer. PCR was performed according to the protocol and guideline provided by manufacturers under control conditions. Ct values below 38 were considered positive and viral load was inversely proportional to Ct value.

To calculate and find out correlation between Ct value and severity of disease, the dengue severity criteria was set according to guideline of World Health Organization which define dengue severity based on clinical sign and symptoms such as plasma leakage leading to shock or fluid accumulation, severe bleeding, organ impairment like hepatitis, cardiac impairment and impaired consciousness [11].

RESULTS

The clinical work of present study was completed in October 2025. The results of current study showed that among these 278 tested samples, 28 were positive for dengue virus by qRT-PCR. These patients show mild to severe clinical manifestation. Of the 28 confirmed dengue cases, DENV-2 was most prevalent serotype 16/28, followed by DENV-1 8/28, DENV-3 3/28 and DENV-4 was found to be least prevalent i.e. 1/28. Seasonal distribution analysis showed highest positivity was observed in the months of August and September with 7 and 10 positive cases respectively. This high positivity ratio may be due to moon soon and heavy rainfall.

The results of present study also showed that the mean Ct value for DENV-1, DENV-2, DENV-3 and DENV-4 were 27.8 ± 4.6, 23.9 ±3.5, 28.1 ±5.0 and 28.4 ±4.4 respectively. It was observed that individuals infected with serotype DENV-2 had significantly lower Ct value which in turn indicated a

higher viral load. A total of 9 patients fell into category of severe dengue, 5 of them showed plasma leakage, 3 with raised liver function test together with respiratory stress and 1 with impaired consciousness. Remaining 19 positive patients were found to be non-severe cases with mild to no symptoms. Among the 9 severe cases, 7 were associated with DENV-2 serotype and 2 with serotype DENV-1. Severe dengue cases demonstrated significantly lower mean Ct 21.6 ± 3.2 as compared to non-severe cases with mean Ct value 26.3 ±3.5.

It was found that platelet count was inversely proportional with viral load which indicated patients with higher viral load were suffering from severe thrombocytopenia. The patient with lower Ct value and higher viral load showed severe clinical presentation such as thrombocytopenia and leukopenia, raised liver function test, more specifically increased Alanine transaminase, persistent fever and severe headache, body and eyes pain and prolonged coagulation parameters.

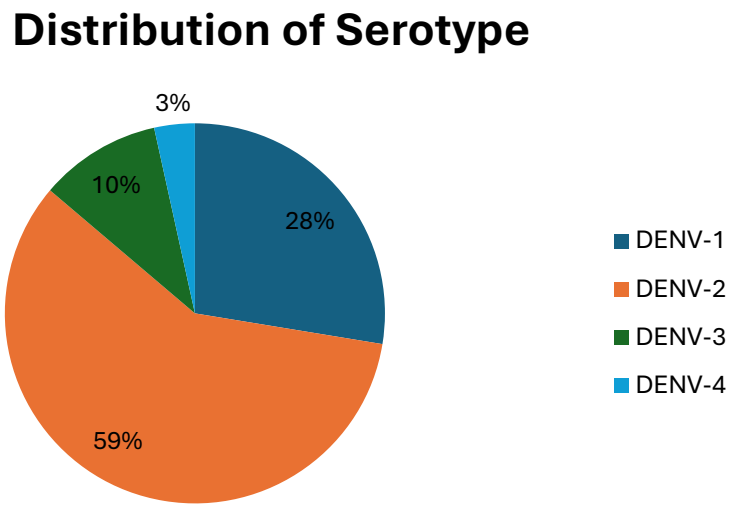


Figure 1: Pie chart presenting distribution of Dengue virus serotype.

Table 1: Seasonal distribution of negative and positive cases

Sr. no	Month	Total test	Negative	Positive
1.	March	12	12	00
2.	April	09	09	00
3.	May	24	23	01
4.	June	31	28	03
5.	July	36	33	03
6.	August	52	45	07
7.	September	67	57	10
8.	October	47	43	04

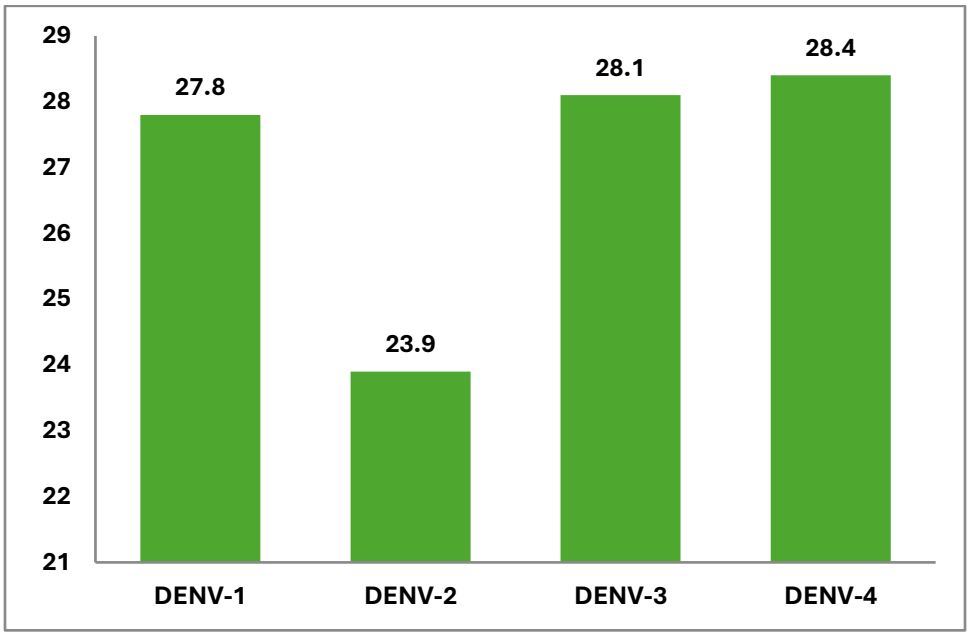


Figure 2: Presenting mean Ct value of Dengue serotypes

DISCUSSION

The outcomes of our current study highlight the predominance of DENV-2 followed by DENV-1 serotype among the tested patients during recent dengue outbreak in studied area, consistent with regional and national reports [12]. DENV-2 has been associated with higher viral load as compared to other serotypes and increased risk of severe illness, this might be due to serotype specific pathogenicity and virulence factor [13].

Our results showed that there was a significant association between lower Ct value and increased disease severity. It has been observed that patients with lower Ct value show more severe symptoms such as thrombocytopenia, high grade fever, prolonged coagulation parameters, muscle pain and raised Liver function test than those with higher Ct value with mild sign and symptom. This relationship highlights the utility of qRT-PCR diagnostic as well as prognostic indicators for dengue viruses. A study in Vietnam showed similar results indicating higher viral load increases the severity of disease, long stay in hospitalization and plasma leakage [14]. Our study revealed that the higher numbers of positive cases were reported in the month of August and September right after the spell of heavy moon soon season. Previous research has also indicated that heavy rainfall influences the survival of larva of Ades Egpty, primary vector for dengue virus which may facilitate the wide spread of dengue infection [15].

In conclusion, DENV-2 serotype was found to be the most prevalent serotype during 2025 dengue outbreak followed by DENV-1, DENV-3 and DENV-4 in studied region. Lower Ct value or higher viral load was directly proportional to severity of disease particularly in patients infected with DENV-2. Serotype specific quantitative real time polymerase chain reaction is gold standard diagnostic tool to assess and monitor the severity of disease.

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Ethics Statement: The study was approved by ethical review committee of Allama Iqbal Teaching Hospital Dera Ghazi Khan under letter number AITH-209-25.
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Data Availability: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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