Original Article

# Association of clinical features and hematological parameters with serotype-specific dengue virus infection

Raima Kalhoro, Safiya Javed, Piriha Abbasi, Mozna Talpur, Aftab Abbasi, Amin Fahim

Department of Pathology, Isra University, Ghulam Muhammad Mahar Medical College
Hyderabad, Pakistan

**Objective:** To identify the relationship of common serotypes of dengue virus in our region with hematological changes in dengue patients.
**Methodology:** This observational study was conducted at Isra University Hospital, Hyderabad and included 100 cases of dengue fever. Dengue virus antibodies were confirmed by using dengue virus rapid test. Serotypes of virus were confirmed by immunofluorescence test. Hematocrit (HCT), total leukocyte count (TLC) and platelet count were determined.
**Results:** The association of majority of common clinical presentation with dengue serotypes was found as non-significant except bleeding, which was found significant. The association of TLC was found significantly associated with severity of serotype. There were only two cases of serotype III and both cases were found with decreased TLC. Thrombocytopenia was significantly associated with serotype. However no significant difference was found between hematocrit values and serotype of dengue virus.
**Conclusion:** Bleeding was only clinical presentation associated significantly with dengue serotypes. The low platelets count and TLC were also significantly associated with severity of dengue serotype. Patients with serotype III had severe thrombocytopenia.
**Keywords:** Dengue virus, serotype, thrombocytopenia, total leukocytes count.

## INTRODUCTION

Dengue virus (DENV) is a positive sense, single stranded RNA enveloped virus that comprises of four serotypes (DENV 1, 2, 3 and 4).[1] Infection occurs due to the bite of the mosquito.[2] About 100 countries are declared as endemic for dengue fever by WHO.[3] The annual infection rateis nearly 50 to 100 million and among these approximately 500,000 require hospitalization every year.[4,5] The fatality is common in children and young adults and reaches upto 5% every year.[6] Two outbreaks of dengue have been reported in Pakistan in year 1994 and 2005.[7]

Serotypes type 2, 3 and 4 are seen in Pakistan.[8, 9] All four serotypes are serologically related, but antigenically distinct.[10] They produce a spectrum of clinical illnesses ranging from a classical dengue fever (DF) to severe and potentially fatal complications known as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).[11] Dengue fever is marked by a sudden onset of high grade fever, severe headache and retro-ocular pain and myalgia. The distinctive characteristics of DHF and DSS consist of hemorrhagic manifestations, plasma leakage, and profound shock.[12,13]

In 2011, dengue outbreak was reported from Lahore, Pakistan andaccording to Health Department, more than 23,000 people were hospitalized, of which at least 365 people died due to DHF and DSS. DENV-2 and DENV-3 and a single case of DENV-4 were reported in the 2011 dengue outbreak.[14] This present study was carried out to identify the relationship of common serotypes of dengue virus in our region with hematological changes in these patients.

## METHODOLOGY

This observational study was conducted at Isra University Hospital, Hyderabad and Liaquat University of Medical and Health Sciences during July to December 2015. The patients were selected through convenience sampling and 100 patients of suspected dengue fever were included, which were calculated through the formula for sampling for proportions. The patients already suffering from hemorrhagic diathesis due to other causes, P.U.O, patients on medication including steroids` and patients with viral infections other than dengue were excluded from the study.

About and 2.5 ml in Gel tube for serotyping and 3ml blood in EDTA tube was collected for hematocrit (HCT), platelets count and total leukocyte count (TLC), which were performed on automated hematology analyzer (Sysmex XN550). Dengue virus antibodies were confirmed by using dengue virus rapid test. Serotypes were confirmed by immunofluorescence test, antibodies of virus were stained with fluoresce labeled anti-human antibodies and antigen antibody complex

appeared coarse granular structures under Fluorescence Microscope.

**Statistical Analysis:** The data were analyzed using SPSS version 21.The tests of significance were applied as per type of variables. Chi square test was used for normality of data. p<0.05 was considered as significant.

## RESULTS

The association of majority of common clinical presentation with dengue serotypes was found as non-significant except bleeding which was found significant (Table 1). Majority of the patients were seen with normal WBC count (65%) while 35% cases were noted with leukocytopenia having WBC count $1\text{-}4 \times 10^9$/L. Thrombocytopenia was seen in the 47% cases with platelets count between $51\text{-}100 \times 10^9$/L, followed by 23% with severe thrombocytopenia between $1\text{-}50 \times 10^9$/L, 21% were found with thrombocytopenia between $101\text{-}150 \times 10^9$/L while only 9% cases were found with more than $> 150 \times 10^9$/L platelets.

**Table 1: Association of Clinical presentation with serotypes (N = 100).**

| Clinical Presentation | Dengue Serotypes | | | |
|---|---|---|---|---|
| | I (n = 79) | II (n = 19) | III (n = 02) | P-value |
| Fever = 91 | 70 | 19 | 02 | 0.21 |
| Body ache = 65 | 50 | 13 | 02 | 0.09 |
| Headache = 55 | 39 | 15 | 01 | 0.24 |
| Anorexia = 45 | 28 | 15 | 02 | 0.12 |
| Vomiting = 15 | 08 | 06 | 01 | 0.72 |
| Rashes = 10 | 05 | 04 | 01 | 0.50 |
| Bleeding = 05 | 00 | 03 | 02 | 0.01 |

**Table 2: Association of Total leukocyte count with serotypes (N = 100).**

| TLC Count | Dengue Serotypes | | | | |
|---|---|---|---|---|---|
| | I (n = 79) | II (n = 19) | III (n = 02) | P-value | Chi Square Value |
| $1\text{-}4 \times 10^9$/L = 35 | 25 | 09 | 01 | 0.05 | 2.110 |
| $> 4 \times 10^9$/L = 65 | 54 | 10 | 01 | | |

The association of TLC was found significantly associated with severity of serotype. There were only two cases of serotype III and both had decreased TLC (p < 0.05) (Table 2). Thrombocytopenia was significantly associated with serotype (Table 3).

**Table 3: Association of Platelets count with serotypes (N = 100).**

| Platelets Count | Dengue Serotypes | | | | |
|---|---|---|---|---|---|
| | I (n = 79) | II (n = 19) | III (n = 02) | P-value | Chi Square value |
| $1\text{-}50 \times 10^9$/L = 23 | 12 | 09 | 02 | 0.001 | 18.798 |
| $51\text{-}100 \times 10^9$/L = 47 | 39 | 08 | 00 | | |
| $101\text{-}150 \times 10^9$/L = 21 | 19 | 02 | 00 | | |
| $> 150 \times 10^9$/L = 09 | 09 | 00 | 00 | | |

However, no significant difference was found between hematocrit values and serotype (p = 0.76) (Table 4).

**Table 4: Association of Hematocrit with serotypes (N = 100).**

| Hematocrit | Dengue Serotypes | | | |
|---|---|---|---|---|
| | I (n = 79) | II (n = 19) | III (n = 02) | P-value |
| Hematocrit | 48 ± 4.2 (%) | 47 ± 5.4 (%) | 45 ± 3.8 (%) | 0.76 |

## DISCUSSION

Over the period of last 30 years, dengue fever is trending towards as emerging pandemic and more than two third of cases of occurs in Asia, Western Pacific region, America, Middle East and Africa.[15,16] Halsey et al reported that musculoskeletal and gastrointestinal manifestations were common in individuals infected with DENV-3 while respiratory and cutaneous manifestations were more pronounced in DENV-4 infected individuals.[17] Individuals infected with DENV-2 had fever, headache, retro-orbital pain, body ache, vomiting, red eye but it can cause severe disease than others, and the individuals infected with DENV-1 had fever, headache, retro-orbital pain, vomiting, red eye, and mild hemorrhage.[17]

In present study, we found that patients who were infected with DENV-1 and DENV-2 had fever headache, body ache, anorexia, vomiting, and rashes, while patients infected with DENV-3 had fever, headache, body ache, anorexia, vomiting, rashes and mucocutaneous bleeding. Other reports had similar findings.[18,19]

In present study, severity of thrombocytopenia was significantly associated with severity of serotype with p-value 0.001. About 47% of the cases were found with platelets count between $51\text{-}100 \times 10^9$/L, whereas 23%

were found with severe thrombocytopenia with platelets between 1-50 × 109/L, while 21% of patients were found with platelet count between 101-150 × 109/L and only 9% cases were found with more than 150 thousand platelets. Khan et al reported that out of 250 patients 82 patients were seen with normal platelet count, 5 patients were found with severe thrombocytopenia (< 25,000/μl), 106 patients with moderately low platelet count (25,000/μl-100,000/μl) and 57 cases with border line thrombocytopenia (100,000/μl-150,000/μl).[20]

Mahmood et al reported that in all 43 DHF patients had platelet count ranging between 1-100 × 109/L which is inconsistent with present study.[21] Yung et alreported there was significant serotype specific differences for platelet count with DENV-2 cases having the lowest platelet count with a median of 114 × 109/L compared with DENV-1(128 × 109/L) and DENV3 (141.5 × 109/L) cases (P < 0.01).[22]

In an international study, Fried et al found that there was no significant difference in the rates of DHF by serotype, whereas DENV-2 was significantly associated with increased odds of DHF grade-1 compared to DF.[23]×Present study is inconsistent with Fried et al[23] and Yung et al.[22] Yung et al reported that there was no significant change in hematocrit in serotype of DENV cases as DENV-143.8 (26.5-74.5, present study is consistent with this study.[22]

## CONCLUSION

Bleeding was only clinical presentation associated significantly with dengue serotypes. The low platelets count and TLC were also significantly associated with severity of dengue serotype. Patients with serotype III had severe thrombocytopenia.

**Author Contributions:**
Conception and design: Raima Kalhoro, Safiya Javed, Aftab Abbasi.
Collection and assembly of data: Piriha Abbasi, Mozna Talpur, Raima Kalhoro.
Analysis and interpretation of data: Safiya Javed, Amin Fahim.
Drafting of the article: Safiya Javed, Aftab Abbasi.
Critical revision of article for important intellectual content: Piriha Abbasi, Aftab Abbasi.
Statistical expertise: Mozna Talpur, Amin Fahim.
Final approval and guarantor of the article: Raima Kalhoro
**Corresponding author email:** Amin Fahim: draminfahim@agmail.com
**Conflict of Interest:** None declared.
Rec. Date: Jun 24, 2021 Revision Rec. Date: Sep 23, 2021 Accept Date: October 14, 2021

## REFERENCES

1. Rosales-Munar A, Alvarez-Diaz DA, Laiton-Donato K, Peláez-Carvajal D, Usme-Ciro JA. Efficient Method for Molecular Characterization of the 5'and 3'Ends of the Dengue Virus Genome. Viruses, 2020; 12: 496-505.

2. Onyango MG, Ciota AT, Kramer LD. The Vector-Host-Pathogen Interface: The Next Frontier in the Battle Against Mosquito-Borne Viral Diseases? Frontiers Cellular Infect Microbiol. 2020; 10: 547-553.

3. Wongsawat J, Vivong N, Suttha P, Utayamakul S, Aumpornareekul S, Chewcharat A, et al. Zika Virus Disease Comparing Children and Adults in a Dengue-Endemic Setting. Am J Trop Med Hyg. 2020; 104: 557-563.

4. Kaushik S, Dar L, Kaushik S, Yadav JP. Identification and characterization of new potent inhibitors of dengue virus NS5 proteinase from Andrographispaniculata supercritical extracts on in animal cell culture and in silico approaches. J Ethnopharmacol. 2021; 267: 113541.

5. Panya A, Sawasdee N, Songprakhon P, Tragoolpua Y, Rotarayanont S, Choowongkomon K, et al. A Synthetic Bioactive Peptide Derived from the Asian Medicinal Plant Acacia catechu Binds to Dengue Virus and Inhibits Cell Entry. Viruses, 2020; 12: 1267-79.

6. Wellekens K, Betrains A, De Munter P, Peetermans W. Dengue: current state one year before WHO 2010–2020 goals. Acta Clinica Belgica. 2020; 1: 1-9.

7. Khetpal A, Godil A, Alam MT, Adam AM, Mallick MS, Abbas MA, et al. Role of C-reactive proteins and liver function tests in assessing the severity of dengue fever. J Pak Med Assoc. 2020; 1: 1-7.

8. Khan NU, Danish L, Khan HU, Shah M, Ismail M, Ali I, et al. Prevalence of dengue virus serotypes in the 2017 outbreak in Peshawar, KP, Pakistan. J Clin Lab Anal. 2020; 34: e23371-76.

9. Khan E, Prakoso D, Imtiaz K, Malik F, Farooqi JQ, Long MT, et al. The Clinical Features of Co-circulating Dengue Viruses and the Absence of Dengue Hemorrhagic Fever in Pakistan. Front Public Health, 2020; 8: 287-89.

10. Hou J, Shrivastava S, Loo HL, Wong LH, Ooi EE, Chen J. Sequential immunization induces strong and broad immunity against all four dengue virus serotypes. NPJ Vaccines, 2020; 5: 1-11.

11. Ghimire N, Paudel D, Yadav MK, Rana S, Chaudhary NK, Rauniyar NK, et al. Study of Clinical and Laboratory Profile in Patients With Dengue Fever at A Tertiary Care Center in Central Nepal. Nepal Med Coll J. 2020; 3: 1-8.

12. Duguma D, Rueda LM, Debboun M. Mosquito-Borne Diseases. In Mosquitoes, Communities, and Public Health in Texas. 2020 Jan 1 (pp. 319-337). Academic Press.

13. Kathiriya JB, Shah NM, Patel JS, Javia BB, Tajpara MM, Ghodasara SN, et al. Epidemiological surveillance of Dengue fever: An overview. Int J Vet Sci Anim Husb. 2020; 5: 1-10.

14. Ali A, Ahmad H, Idrees M, Zahir F, Ali I.Circulating serotypes of dengue virus and their incursion into non-endemic areas of Pakistan; a serious threat. Virol J. 2016; 13: 1-8.

15. Herencia JS. Lessons Learned and Recent Advances in Dengue Research. Dengue Fever in a One Health Perspective, 2020.

16. Sood SK, Sood V, Mahajan I. An intelligent healthcare system for predicting and preventing dengue virus infection. Computing, 2021: 1-39.

17. Halsey ES, Marks MA, Gotuzzo E, Fiestas V, Suarez L, Vargas J, et al. Correlation of serotype-specific dengue virus infection with clinical manifestations. PloS Negl Trop Dis. 2012; 6: e1638:1-10.

18. Butt N, Abbassi A, Munir S, S. Ahmad S, Sheikh Q. Haematological and Biochemical Indicators for the Early Diagnosis of Dengue Viral Infection. J Coll Physicians Surg Pak. 2008; 18: 282-85.

19. Ahmed F, Hussain Z, Ali Z. Clinical and hematological profile of patients with dengue fever. J Med Sci. 2014; 22: 17-20.

20. Khan MU, Rehman R, Gulfraz M, Latif W. Incidence of thrombocytopenia in seropositive dengue patients. Int J Med Sci. 2014; 6: 113-6.

21. Mahmood K, Jameel T, Aslam HF, Tahir M. Incidence of dengue haemorrhagic fever in local population of Lahore, Pakistan. Biomedica 2009; 25: 93-6.

22. Yung CF, Lee KS, Thein TL, Tan LK, Gan VC, Wong JG, et al. Dengue serotype-specific differences in clinical manifestation, laboratory parameters and risk of severe disease in adults, Singapore. Am J Trop Med Hyg. 2015; 92: 999-1005.

23. Fried JR, Gibbons RV, Kalayanarooj S, Thomas SJ, Srikiatkhachorn A, Yoon IK, et al. Serotype-specific differences in the risk of dengue hemorrhagic fever: an analysis of data collected in Bangkok, Thailand from 1994 to 2006. PLoS Negl Trop Dis. 2010; 4: e617:1-6.