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Serological Distribution and Hematological Profiling of Dengue Virus Infection at Hyderabad and Adjoining Areas: A Cross-Sectional Study

Raja Rehan[1], Beenish Khanzada[1*], Saba Naz[2], Zaib Un Nisa Mughal[3] and Ghulam Rasool Rind[1]

[1]Institute of Biochemistry, University of Sindh, Jamshoro, Pakistan
[2]Institute of Chemistry, University of Sindh, Jamshoro, Pakistan
[3]Department of Physiology, University of Sindh, Jamshoro, Pakistan

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***Corresponding Author:**
Beenish Khanzada
Institute of Biochemistry, University of Sindh, Jamshoro, Pakistan
beenish@usindh.edu.pk

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ABSTRACT

Pakistan appears to be a dengue endemic area with seasonal outbreaks and a high spike every year. **Objectives:** To investigate the period prevalence (positivity rate) of dengue viral infection (DVI) among suspected cases in the hospital and other factors related to DVI in Hyderabad district, Sindh, Pakistan, between August and December 2025. **Methods:** A cross-sectional study to find out the period prevalence of DVI through a hospital survey and specially designed Questionnaires including 20-60 years' age patients complaining of dengue-specific symptoms. Dengue NS1 antigen test, IgM, IgG, and CBC were performed, and results were interpreted using the chi-square test via SPSS version 22.0. **Results:** Out of 544 patients, 255 cases were dengue positive. 244 (95%) were found to be dengue NS1 antigen positive, 8 (3%) were IgG positive, and 3 (1%) were positive with IgM. Age and gender revealed no significant statistical association with DVI. However, more males (49%) in the age range of 20-30 years were majorly affected by the dengue virus. Seasonal variation study depicted October as the month badly hit by DVI with a major surge in Dengue, i.e., 140 positive cases. The Hyderabad main city area and Qasimabad were majorly affected by DVI. The most common symptoms observed in dengue-positive cases included muscle and joint pain, vomiting, headache, and nausea accompanied by fever. **Conclusions:** The research finds that dengue infection is in a significant upsurge in Hyderabad, and the majority of the positive cases are in the early acute stage (NS1-positive).

INTRODUCTION

A visible shift in climate and subtropical region enclosure has increased the vulnerability of Pakistan to vector-mediated life-threatening diseases like dengue viral infection (DVI) [1]. Dengue virus is categorized in the RNA virus class and belongs to the Flaviviridae family with four distinct serotypes named as DENV 1 to DENV 4. Dengue, if untreated, may lead to severe complications like dengue shock syndrome (DSS) or dengue hemorrhagic fever (DHF) [2]. Dengue infection is characterized by sudden fever onset with myalgia, arthralgia, severe headache, and skin rash [3]. Fever may last 2 7 days with possible abrupt onset, whereas the virus incubation period is usually recorded to be 3 14 days [2]. Complete blood count sounds crucial for the diagnostic process of dengue infection, with thrombocytopenia as a commonly observed feature [4]. Although the disease is self-limiting and recovery is possible within a week, it has crossed the annual mortality toll of >20,000 [5]. According to the World Health Organization estimates, annually, 390 million cases have been reported, with 70% of the disease burden shared by Asia only [6]. As of November 25, 2021, Pakistan is reported to have recorded 48,906 dengue cases, along with 183 fatalities; Punjab had the highest disease frequency [7]. Dengue may be rising in Pakistan due to several factors,

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such as socioeconomic and climate shift factors, unplanned urbanization, more use of disposable items serving as mosquito breeding places, insecticide resistance, poor infrastructure, variations related to demographics, and inadequate sanitation conditions throughout the country [8]. The first outbreak of dengue fever was reported in 1982, with a variety of serotypes involved. According to the WHO and regional surveillance reports, several dengue serotypes (DENV 1- DENV 4) have been in existence in Pakistan, and the distribution of the serotypes has varied by both year and place. New information provided by CDC and peer-reviewed sources (2022-2025) must be used in place of older articles dated 2006 [9]. Likewise, multiple serotypes had been observed in Lahore during the 2008 disease outbreak, with a higher prevalence of DHF [2]. AS far as Sindh province is concerned, recent floods have likely contributed to stagnant water resources and thus to disease spread. Very few studies have targeted Hyderabad Sindh for finding out dengue prevalence in the district and associated risk factors. Arain *et al.* has reported a very low prevalence of only 7.2% during 2010 2017 at Hyderabad [7]. However, more recently, Chang *et al.* reported a study on 120 dengue cases at a civil hospital in Hyderabad during August November 2024, where the authors detected alterations in hematological profile and platelet variations [10]. This study theorized that the prevalence of dengue in the suspected patients in the period would be high and that the NS1 antigen would be the dominant dengue infection marker of acute infection. This study also postulated that thrombocytopenia and leukopenia would be much more common in dengue-positive cases than in dengue-negative cases. This study hypothesized that NS1 would be the most common acute biomarker and that thrombocytopenia/leukopenia would be significantly correlated with dengue positivity.

There is a gap in recent post-flood serological and hematological evidence on dengue in Hyderabad, Sindh. This study aimed to identify the present positivity rate, serological distribution (NS1/IgM/IgG), and CBC changes in suspected patients (August, 2025-December 2025), which provides epidemiological surveillance data and diagnostic information.

METHODS

A cross-sectional study was conducted at four different hospitals of the district Hyderabad Sindh to determine point prevalence of dengue viral infection during August 2025 to December 2025, including both inpatient and outpatient, aged 20 60 years, complaining of fever >over 38ºC for 3 10 days, and presenting with either of the dengue-related symptoms like fever, headache, rash, bleeding, body pain, vomiting, etc. The study was commenced following ethical approval from the ethical committee of the Institute of Biochemistry, University of Sindh, Jamshoro (Ref. number: IOB/287/2025). The formula $n=Z^2p(1-p)/d^2$ was used to determine the sample size with a 5% margin of error, the expected prevalence of dengue being 40% (calculated based on a previous local study), the 95% confidence level (Z=1.96), and p=0.4. This provided the minimum number of 369 participants; to ensure accuracy, the study recruited 544 participants. Oral and written Informed consent was obtained from all patients. Specially designed Questionnaires were given to patients for information regarding demographics, medical history, associated risk factors, and symptoms. Patients aged less than 20 years and >60 years were excluded. Patients having chronic liver disease, Hematological malignancies, or chronic illness requiring the use of steroids or immunosuppressants were also excluded from the study. Inclusion criteria were age 20–60 years, fever >38°C lasting 3–10 days, plus at least two of the following: headaches, myalgia, arthralgia, rash, vomiting, or bleeding. Exclusion criteria were chronic liver disease, hematological malignancies, or chronic steroid/immunosuppressant use. Participants were recruited using consecutive sampling (all eligible patients presenting during the study period were invited to participate). This approach was chosen to minimize selection bias, though convenience sampling was not used. Serology distribution of DVI was checked using the Rapid Chromatographic Immunoassay kit (The Hangzhou Tongzhou Biotechnology China ICT Kit) for the detection of Dengue AG Test. A blood sample (5 ml) was collected for analysis of dengue virus protein (NS1 antigen via ICT Method), antibody (IgG & IgM), and Complete blood counts (using Nihon Codon CBC analyzer). Dengue NS1 Antigen test was done, which allows early detection of NS1 antigen that even appears in blood on the first day of fever before antibodies start to appear. Moreover, an antibody (IgG & IgM) test was also performed to see the frequency of secondary DVI infection.

All the findings were recorded, and results were analyzed statistically using Statistical Package for the Social Sciences (SPSS) software version 22.0 (IBM). Mean ± SD was calculated for CBC Parameters. Chi-square test was employed in finding the association between demographic factors (age and gender) with Dengue infection, with a p-value of <0.005 considered significant.

RESULTS

Chi-square analysis showed no significant association between gender and dengue positivity (χ^2 = 1.13, df = 1, p=0.287). However, age was significantly associated with dengue positivity (χ^2 = 18.02, df = 3, p<0.001), with the highest proportion of positive cases in the 20–30-year age group (55%). The most targeted age group affected by DVI

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was found to be the 20-30 years range group, with the highest number (55%) of dengue positive cases in this age group with a non-significant association (p-value>0.005) (Table 1).

Table 1: Demographic Factors Associated with Dengue Virus Infection (DVI)

Parameters	Total Patients (n=544)	Dengue Positive, n (%)	Dengue Negative, n (%)	χ^2 value	df	p-value*
Gender						
Male	350	170 (49%)	180 (51%)	1.13	1	0.287
Female	194	85 (44%)	109 (56%)			
Age (Years)						
20-30	220	121 (55%)	99 (45%)	18.02	3	<0.001
31-40	182	88 (49%)	94 (51%)			
41-50	98	31 (32%)	67 (68%)			
51-60	44	15 (34%)	29 (66%)			

*χ^2 test; statistical significance set at p<0.005

Among all positive cases, 244 were dengue NS1 Antigen positive, whereas only 8 and 3 patients were found positive for IgG and IgM, respectively, showcasing the lower prevalence of dengue secondary infection (Figure 1).

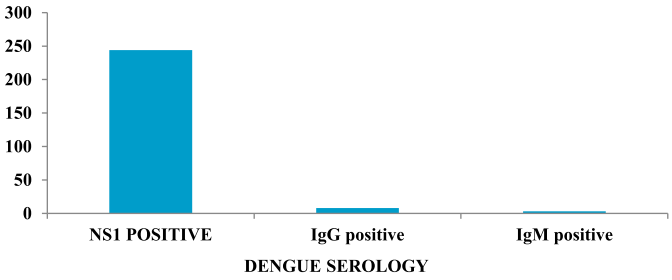

Figure 1: Serology Distribution of Dengue Positive Cases

Month-wise prevalence analysis suggested September and October as the main months that were hit badly by dengue in the Hyderabad region, with 58 and 140 positive dengue patients, respectively (Figure 2).

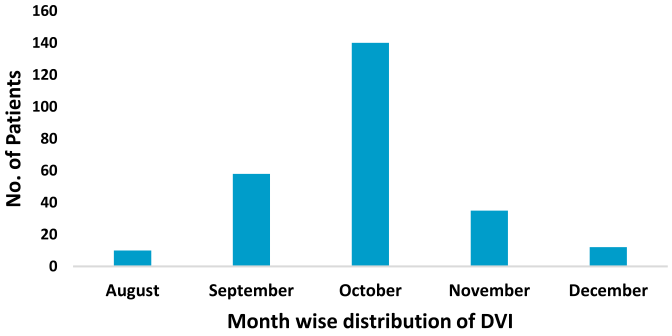

Figure 2: Month-wise Distribution of DVI

Area-wise study revealed that the Hyderabad main city area and Qasimabad were majorly affected by DVI with 90 and 80 positive dengue patients, respectively (Figure 3).

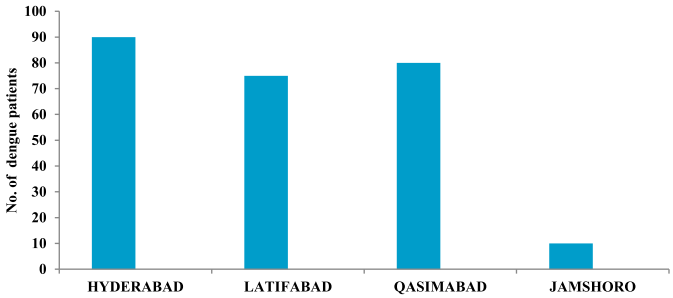

Figure 3: Area-Wise Distribution of DVI

Symptoms shown by dengue positive patients included 143 patients with muscle pain,98 patients with joint pain, and others suffering from other symptoms like fever, vomiting, headache, nausea, etc. (Table 2).

Table 2: Symptoms Observed in Dengue Positive Cases (n=255)

Sr. No.	Symptom	n (%)
1	Fever	219 (85.9%)
2	Myalgia	143 (56.1%)
3	Arthralgia	98 (38.4%)
4	Headache	170 (66.7%)
5	Vomiting	21 (8.2%)
6	Nausea	16 (6.3%)
7	Skin rash	30 (11.8%)

Evaluation of CBC parameters depicted a mild drop in RBCS and an increase in lymphocytes. Platelets and WBCS were seen to be decreased in dengue-positive cases as compared to negative ones (Table 3).

Table 3: CBC Parameters in Dengue Virus Infection

CBC Parameters	Ns1 Positive, Mean ± SD	Ns1 Negative, Mean ± SD
Hemoglobin (g/dl)	12.8 ± 1.63	13.4 ± 2.24
Platelets (m/cmm)	172.3 ± 52.3	316.4 ± 107
RBC (m/cmm) ($10^{\wedge 6}$/µL)	4.75 ± 0.83	4.8 ± 0.72
WBCs ($10^{\wedge 3}$/UL)	4.70 ± 1.90	7.36 ± 3.55
Neutrophils (%)	34.0 ± 22.5	63.2 ± 16.4
Lymphocytes (%)	54.8 ± 22.1	25.4 ± 12.9

DISCUSSION

DVI presentation varies area-wise; it depends on different factors such as age, gender, immune status, and particular strain of virus affecting the patients [4, 11]. In current study, around 47% Point prevalence (out of 544 patients) was seen at Hyderabad Sindh region, which shows a steady increase in DVI prevalence in comparison to a study of 2006 2007 at the same Hyderabad area, which depicted around 40% positive dengue cases [10]. The current study may have the limitation of molecular studies; however, IGM was only detected in 3 patients, which may not be evident during the early infection phase. For the majority of negative dengue cases found in our study, they may have been affected by other vector-borne epidemics like chikungunya or Zika virus, etc., [12] although not verified in

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our research. Moreover, males were found to be more affected by dengue infection in our study, which is similar to previously carried out research on dengue, which reports that the more outside exposure of males may be a valid reason for the increased infection rate in males compared to females. The most affected age group found in our study was the 20-30 age group, similar to the already reported 16-30 most frequent age group reported by previous studies done at Hyderabad and Haripur region, as well as India-based studies [13-15]. The weather has a great influence on the spread of DVI. Considering the seasonal atmosphere of Hyderabad, the area may be divided into the monsoon season (August to September) and the post-monsoon season (October to December). These all-month temperatures provided an ideal atmosphere for Aedes mosquitoes to grow and reproduce. According to previous reports, Hyderabad records around 200 mm annual rainfall with an annual mean temperature of 17-28°C. Post-moon soon period also provided low temperatures, i.e., 20°C, which particularly aided in the fertilization of these mosquitoes. Previous studies support that low temperature was actually linked with more spread of DVI [7, 16]. This is in agreement with previous yearly prevalence of DVI at Hyderabad, where in 2010, 2013, and 2016, flooding increased the prevalence of dengue infection [13, 17]. This study found that 8 cases had IgG antibodies and 3 had IgM antibodies. The problem is that we did not take blood samples at times or use a reliable method to compare IgM and IgG antibodies. This means we cannot say for sure if these people had a secondary infection. If someone has IgG antibodies, it might mean they were exposed to the infection before. Without more testing, we cannot say for certain if they had a secondary infection or if it was more severe. The IgG antibodies alone do not give us information about secondary infections and how serious they might be. As per current results, the Hyderabad main city area was found to be mainly affected by DVI. It may be due to a higher population rate, congested streets, inadequate sanitation, accumulated water, spare tyres, and garbage heaps, etc. [18]. Previous researchers have declared some other factors contributing to an area's susceptibility to DVI, such as rainfall intensity, vector control services performance, and urbanization [19]. As per serology distribution, the Dengue NS 1 test came out to be an early indication and most effective for revealing DENV. We did not use a validated serological algorithm (such as IgM/IgG ratio or paired acute/convalescent sera) to distinguish primary from secondary infection. The low number of IgG-positive cases suggests possible past infection, but without confirmatory criteria, claims about primary versus secondary infection cannot be made [20]. Clinical blood profile showed dengue characteristic features similar to previous studies, such as a drop in platelets, WBCs, RBCs, and Neutrophils were seen in dengue positive cases as compared to negative cases. These results are consistent with previous findings where DVI has been associated with thrombocytopenia, lymphopenia, and leucopenia, and all these CBC alterations serve as disease severity potential indicators [21].

This research has some limitations, such as being cross-sectional (precluding causal statements) and using a diagnosis of dengue without molecular confirmation (e.g., by PCR). Further, single-time serological tests limit the ability to distinguish primary from secondary infections. Longitudinal studies, larger samples from multiple centers and enhanced diagnostics (e.g., PCR, paired serology) should be applied in future studies to increase the accuracy of diagnosis and disease understanding.

CONCLUSIONS

This study depicts dengue as a significant outbreak affecting more males than females, with the predominantly affected 20 to 30-year-old age group individuals having a high frequency of primary infection at Hyderabad, Sindh, Pakistan. The dengue transmission was found to be affected by seasonal variations, area sanitation, temperature variations, and was more prevalent in populated areas like urban city areas, with a peak in the month of October. Clinical investigations revealed thrombocytopenia and leucopenia as the main elements of the disease severity. Effective control measures, practice of covered water containers, and improving area sanitary conditions are highly suggestive.

Authors' Contribution

Conceptualization: RR
Methodology: RR, GRR
Formal analysis: SN, ZUNM, GRR
Writing and Drafting: RR, BK
Review and Editing: RR, BK, SN, ZUNM, GRR

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

The authors declare no conflict of interest.

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REFERENCES

[1] Channa MA, Memon N. 12. Seasonal Variation in the Prevalence of Larvae of Aedes Aegypti Mosquito in District Hyderabad, Sindh, Pakistan. Pure and Applied Biology. 2020 Apr; 9(2): 1354-63. doi: 10.19045/bspab.2020.90142.

DOI: https://doi.org/10.54393/pbmj.v9i3.1356

[2] Sahar G, Mobeen H, Ghafoor MA, Baghdadi MH, Fatima A. Seroepidemiology of Dengue Fever and the Associated Sociodemographic, Clinical, and Environmental Factors in Lahore, Pakistan. Journal of Pharma and Biomedics. 2025 Jun; 3(1): 83-9.

[3] Liu LT, Huang SY, Lin CH, Chen CH, Tsai CY, Lin PC *et al.* The Epidemiology and Identification of Risk Factors Associated with Severe Dengue During the 2023 Dengue Outbreak in Kaohsiung City, Taiwan. Travel Medicine and Infectious Disease. 2025 May; 65: 102852. doi: 10.1016/j.tmaid.2025.102852.

[4] Ullah W, Izaz M, Nasir A, Miss R, Islam Z, Khan M *et al.* Epidemiology and Risk Factors of Dengue Fever Outbreak in Peshawar, Pakistan: A Hospital-Based Surveillance Study. Indus Journal of Bioscience Research. 2025 May; 3(5): 331-6. doi: 10.70749/ijbr.v3i5.1192.

[5] Najafi S, Jojani M, Najafi K, Costanzo V, Vicidomini C, Roviello GN. West Nile Virus: Epidemiology, Surveillance, and Prophylaxis with a Comparative Insight from Italy and Iran. Vaccines. 2026 Jan; 14(1): 57. doi: 10.3390/vaccines14010057.

[6] Yang X, Quam MB, Zhang T, Sang S. Global Burden for Dengue and the Evolving Pattern in the Past 30 Years. Journal of Travel Medicine. 2021 Dec; 28(8): taab146. doi: 10.1093/jtm/taab146.

[7] Arain SQ, Channa NA, Talpur FN, Memon NH, Sheikh M, Phull AR. Dengue Fever: Prevalence, Risk Factors and Serological Diagnosis in Hyderabad, Sindh, Pakistan. Journal of Fatima Jinnah Medical University. 2024; 18(4): 185-90. doi: 10.37018/AXDS9956.

[8] Shabbir W, Pilz J, Naeem A. A Spatial-Temporal Study for the Spread of Dengue Depending on Climate Factors in Pakistan (2006–2017). BioMed Central Public Health. 2020 Jun; 20(1): 995. doi: 10.1186/s12889-020-08846-8.

[9] Akhtar K. Dengue Infected Patients in the Local Population of Lahore: Dengue Patients in Lahore. Pakistan BioMedical Journal. 2022 Dec: 02-6. doi: 10.54393/pbmj.v5i12.833.

[10] Chang Z, Birmani NA, Chang AH, Jamali L, Chang N, Chang F. Analysis of Clinical and Hematological Findings in Malaria-Infected Patients in Hyderabad, Sindh. Biologia(Lahore). 2024; 70(2): 47-53.

[11] Jamal Z, Humayun F, Haider SA, Hakim R, Hill V, Saeed SY *et al.* Genomic Characterization and Serotype Distribution of Dengue Virus Circulating in Pakistan During 2024. Infection, Genetics and Evolution. 2025 Aug: 105815. doi: 10.1016/j.meegid.2025.105815.

[12] Pescarini JM, Rodrigues M, Paixão ES, Cardim L, De Brito CA, Costa MD *et al.* Dengue, Zika, and Chikungunya Viral Circulation and Hospitalization Rates in Brazil from 2014 To 2019: An Ecological Study. PLos Neglected Tropical Diseases. 2022 Jul; 16(7): e0010602. doi: 10.1371/journal.pntd.0010602.

[13] Qureshi H, Khan MI, Bae SJ, Akhtar S, Khattak AA, Haider A *et al.* Prevalence of Dengue Virus in Haripur District, Khyber Pakhtunkhwa, Pakistan. Journal of Infection and Public Health. 2023 Jul; 16(7): 1131-6. doi: 10.1016/j.jiph.2023.04.021.

[14] Shahid M, Amin I, Afzal S, Fatima Z, Zahid S, Ashraf U *et al.* Prevalence and Molecular Detection of Dengue Virus in the 2013 Outbreak in KPK and Punjab, Pakistan. Pakistan Journal of Zoology. 2017 Jun; 49(3). doi: 10.17582/journal.pjz/2017.49.3.sc4.

[15] Elanchezhiyan C, Gogoi U, Shende V, Basu S, Bandyopadhyay S, Mandal B *et al.* Spatio-Temporal Distribution of Dengue Cases and Vectors Along with the Interrelationship of Environmental and Climatic Factors in the Metropolitan City, Kolkata, India, for 2017-2022: Calling Implications for Vector Control. Emerging Microbes and Infections. 2025 Apr; 14(1): 2493924-. doi: 10.1080/22221751.2025.2493924.

[16] Saeed A, Ali S, Khan F, Muhammad S, Reboita MS, Khan AW *et al.* Modelling the Impact of Climate Change on Dengue Outbreaks and Future Spatiotemporal Shift in Pakistan. Environmental Geochemistry and Health. 2023 Jun; 45(6): 3489-505. doi: 10.1007/s10653-022-01429-z.

[17] Zafar Z, Zubair M, Fahd S. Extreme Weather Events and Their Socioeconomic Impacts: A Remote Sensing-Based Analysis of Flood Damages. Global and Earth Surface Processes Change. 2024 Jun; 1: 100001. doi: 10.1016/j.gespch.2024.100001.

[18] Yani A. The Influence of Environmental Factors on the Development of Dengue Fever. The International Science of Health Journal. 2024 Dec; 2(4): 116-23. doi: 10.59680/ishel.v2i4.1569.

[19] Wang D, Wu X, Chen J, Zhao L. Urbanization, Infrastructure, and Mosquito Thermal Suitability Shape the Causal Effects of Extreme Weather on Dengue Transmission. The Innovation Medicine. 2026 Jan; 4(1): 100188. doi: 10.59717/j.xinn-med.2026.100188.

[20] Ramu ST, Dissanayake M, Jeewandara C, Bary F, Harvie M, Gomes L *et al.* Antibody and Memory B Cell Responses to the Dengue Virus NS1 Antigen in Individuals with Varying Severity of Past Infection. Immunology. 2023 Sep; 170(1): 47-59. doi: 10.1111/imm.13651.

[21] Zeb F, Haleem KS, Almuqbil M, Rashid M, Hussain W, Maqbool F *et al.* Age, Gender, and Infectious Status-Wise Assessments of Hematological Parameters Among Patients with Dengue Infection. Heliyon. 2024 Jul; 10(13). doi: 10.1016/j.heliyon.2024.e34053.