

Serological evidence of dengue fever and its associated factors in patients

Muhammad Salman¹, Nimra Nisar^{1*}, Marium Younas¹, Faisal Iqbal², Hira Afzal¹, Adnan Abdul Majeed¹

¹ Department of Medicine, Hameed Latif Hospital Lahore. ² Department of Medicine, Islam Medical and Dental College, Sialkot

ABSTRACT

Background: Dengue virus (DENV), a viral infection, is transmitted to humans primarily through bites from infected mosquitoes called *Aedes aegypti* mosquitoes and, to a lesser extent by the *Aedes albopictus* mosquitoes. Globally, the incidence of dengue is increasing, with an estimated half of the world's population at risk. This study aimed to evaluate serological indicators of dengue and associated risk factors among patients.

Methods: A cross-sectional study conducted at Hameed Latif Hospital from 15 July 2024 to 30 September 2024. 490 patients were determined using G-POWER software. The study included all patients presenting to the emergency department with symptoms indicative of dengue during the study period. Data were analyzed with SPSS version 25. Frequency tables were presented for quantitative data, and the chi-square test was used for categorical variables (level of significance $p < 0.05$).

Results: The overall seroprevalence of anti-DENV IgG and IgM antibodies was 23.7% and 7.6%, respectively. Of the 490 confirmed cases, 421 were male (58.8%), and 295 were female (41.2%). The highest incidence was observed in the 16–30 age group (42.0%), followed by 31–45 years (25.7%), above 46 years (18.4%), and 0–15 years (13.8%).

Conclusion: Dengue showed substantial seroprevalence, with significant associations with sex and residence. Common clinical manifestations and characteristic hematological abnormalities underscore the importance of early diagnosis, surveillance, and targeted preventive measures.

Keywords: Complete Blood Count, Dengue fever, IgG, IgM, Seroprevalence

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Corresponding Author

Nimra Nisar

Department of Medicine, Hameed Latif Hospital.

Email address: hnisar_verda@yahoo.com

Introduction

Dengue virus (DENV) is a mosquito-borne viral infection transmitted primarily through the bites of infected *Aedes aegypti* mosquitoes and, to a lesser extent, *Aedes albopictus* mosquitoes. Globally, the incidence of dengue is increasing, with an estimated half of the world's population at risk. There are several factors associated with this rise in dengue incidence. Among them, rapid population

expansion, haphazard urban development, poor water storage, and inadequate sanitation contribute to its exacerbation. Warm temperatures and increased rainfall create ideal breeding conditions for *Aedes* mosquitoes, which are the primary vectors of the virus (1).

Each year, around 100–400 million infections occur, most of which are mild or asymptomatic febrile illness to classic DF to the most severe form of illness, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (2). Diagnosing DF is clinically challenging in developing countries

due to a lack of diagnostic infrastructure, often leading to misdiagnoses as malaria, typhoid, or other febrile illnesses (3).

Severe dengue is a major factor in serious illness and death in some Latin American and Asian nations (4). DENV has no specific cure medications. Dengue is found in over 128 countries across Asia, the Americas, the Western Pacific, and Africa. Growing concerns suggest that rising temperatures due to climate change may help the virus spread to areas that are currently free of dengue. This situation arises from inadequate healthcare infrastructure, misdiagnosis, confusion with other febrile illnesses, and insufficient surveillance systems, especially in resource-constrained environments, exacerbating the issue. Underreporting can lead to inaccurate epidemiological data, which is essential for effective disease control and prevention (5).

DVI is now endemic all over Pakistan, with cases peaking after the monsoon season especially among younger individuals (6). Dengue can manifest in three main forms based on severity: mild, severe, and fatal. Studies confirm that the virus is most prevalent in Pakistan following the monsoon season (7). From 1994–2011, the DENV disease caused numerous outbreaks in Pakistan. The infection was first testified in Pakistan in 1982 when 12 patients out of 174 were affected (8). Between 2010 and 2011, Pakistan experienced devastating floods that not only resulted in extensive damage to property and loss of life but also created increased breeding grounds for the dengue virus. Consequently, severe outbreaks were reported, totaling 22,562 dengue cases and 363 fatalities, among which (17493) cases with 290 deaths originated from Lahore, Punjab alone. Notable changes in hematological and biochemical parameters can serve as indicators of disease severity and assist in monitoring patient status (6).

In Pakistan, dengue is mainly regarded as a disease that affects urban and semi-urban areas. In 2019, the country recorded 47,120 confirmed cases of dengue, resulting in 75 fatalities. The Punjab province was responsible for 21% (9,676) of the total dengue cases nationwide, and during that time, there were 16 deaths reported (9).

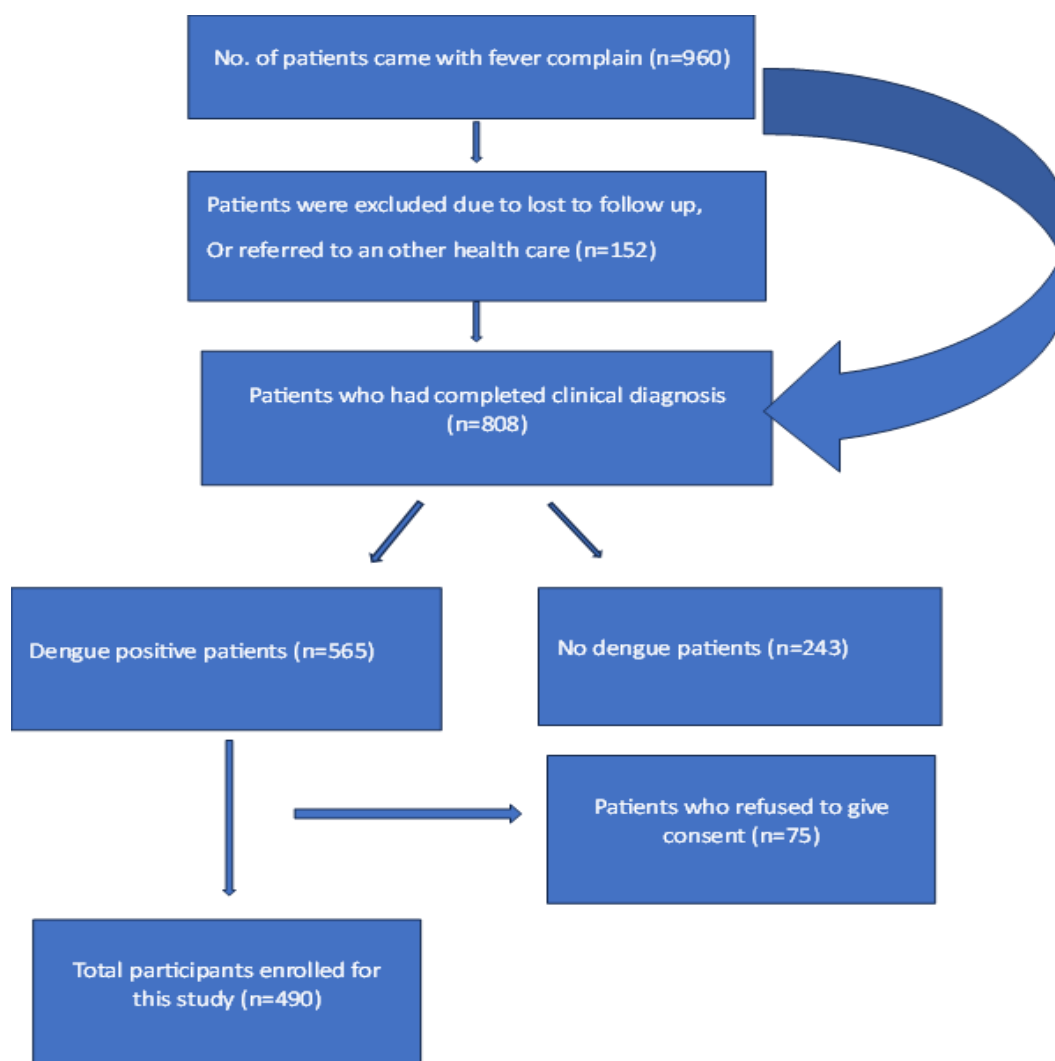
Pakistan has been suffering from dengue outbreaks for decades and increase in dengue cases at alarming rate has been reported by Pakistani officials. Total dengue cases 48,906 with 183 deaths from January 01 to November 25, 2021 and total 47,120, cases of dengue in 2020 including 75 deaths were reported in Pakistan (10). This research aims to support health authorities with data on DENV infections to enhance patient management and strengthen preventive strategies.

Methods

It was a cross-sectional study, conducted from 15 July 2024 to 30 September 2024 with a calculated sample size of 490 by using G-POWER. Patients presenting with acute febrile illness to emergency were recruited. Febrile illness was defined as a body temperature $\geq 38^{\circ}\text{C}$. This study was approved by the institutional review board committee of the hospital with reference number: HLH/IRB/2024-014 dated march 20 2024. Patients with suspected dengue and more than two days of fever, along with at least two symptoms such as headache, retro-orbital pain, rash, myalgia, bleeding, or leukopenia were included. Patients with confirmed bacterial or chronic infections and those with only IgG positivity were excluded from the study. Participants were interviewed using a structured questionnaire focusing on socio-demographic data. The written consent form was taken by patients. After taking the consent form from all the patients, the blood sample (10 mL) from each individual was taken and

divided into vacutainers for hematological, and diagnostic studies. Blood samples for diagnostic studies were centrifuged and serums were stored at 4°C for further analysis within a week. Hematological parameters were performed on the same day. Patients' blood were subjected to dengue test as nonstructural protein 1 (NS1) antigen and dengue specific IgG/IgM antibodies in vitro by standard laboratory procedure as Immuno chromatographic test (ICT) using commercially available SD BIOLINE Dengue Duo rapid test dengue diagnostic kit (Standard Diagnostic Inc; Seoul, Korea) and by Enzyme linked immunosorbent assay

(ELISA) using commercially available kits for NS1 antigen, IgM and IgG antibodies (Diagnostic Automation/ Cortez Diagnostics Inc, California, USA). All samples were analyzed according to manufacturer protocol. The Statistical Package for Social Sciences (SPSS) version 25 was used for the data entry and analysis. Frequency tables were generated for quantitative data. Chi-square test was applied to categorical data to check the association between different parameters. Prevalence was determined by IgM and/or IgG positivity. The level of significance was 5%.



Results

A total of 490 participants were investigated during this study, the median age was 27 years (range: 10–50). Out of these, 103 (21.2%) belonged to ≤15 years of age, 16–25 age group made up 235 (47.9%) of the sample, 86 (17.5%) were 26–35 years, 56 (11.4%) were 36–45 years and 10 (2%) belonged to >45 years. Majority of patients were males comprised 280 (57.1%) and females 210 (42.9%). Urban residents were 329 (67.2%) and rural were 161 (32.8%). with 315 (64.2%) educated and 175 (35.8%) were illiterate. 237 (48.4%) employed while 122 (24.9%) were students, 89 (18.1%) were house wife and 25 (5.1%) patients were farmers. Table 1

IgG and IgM antibodies were detected in 23.7% and 7.6% of the participants, respectively. The prevalence of IgG was greater in males (27.9%) compared to females (18.1%). The age group of 16–25 demonstrated the highest prevalence of IgG (28%). City dwellers and workers had elevated percentages (44.1% and 30.1%, respectively). Gender and urban living displayed notable correlations with IgG positivity ($p < 0.05$). Urban participants had a 0.37 times higher likelihood of testing positive compared to rural residents. Clinically, severe DF was more linked to DHF than dengue shock syndrome (DSS). DHF is characterized by capillary leakage and hemorrhage, whereas DSS signifies its severe phase, distinguished by shock and fluid depletion. The occurrence of anti-DENV IgM seropositivity showed no statistically significant association with age,

gender, location, education, or occupation. Table 2

Patients exhibited a range of clinical features including fever, headache, retro-orbital discomfort, nausea, vomiting, bleeding, and diarrhea. The main symptoms consisted of headache (83.3%), body pain (72.7%), pain behind the eyes (68.5%), nausea (44.2%), and vomiting (19.8%). Table 3

The neutrophils, platelets, leukocytes, and monocytes were significantly lower ($P < 0.05$) in the CBC levels of patients with dengue. Table 4

Table 1: Demographic characteristics of participants

Variables	n (%)
Age (years)	
≤15	103 (21.2)
16-25	235 (47.9)
26-35	86 (17.5)
36-45	56 (11.4)
>45	10 (2)
Gender	
Female	210 (42.9)
Male	280 (57.1)
Residence	
Urban	329 (67.2)
Rural	161 (32.8)
Education	
Educated	315 (64.2)
Illiterate	175 (35.8)
Occupation	
Employee	237 (48.4)
Student	122 (24.9)
Housewife	89 (18.1)
Farmer	25 (5.1)
Others	17 (3.5)

Notes: In Others category, Day laborer, Merchants were included

Table 2: Factors associated with the prevalence of anti-DENV IgG and IgM seropositivity

Variables	anti-DENV IgG n(%) positive	P-value	anti-DENV IgM n(%) positive	P-value
Age (years)				
≤15	19 (18)	0.78	5 (4.9)	0.44
16-25	66 (28)		16 (6.9)	
26-35	23 (26.7)		8 (9.3)	
36-45	6 (10.7)		5 (8.9)	
>45	2(20)		3 (30)	
Gender				
Female	38 (18.1)	0.00*	21 (10)	0.11
Male	78 (27.9)		16 (5.7)	
Residence				
Urban	71 (44.1)	0.00*	21 (13.2)	0.29
Rural	45 (13.6)		16 (4.8)	
Education		0.34		0.33
Educated	69 (22.1)		22 (6.9)	
Illiterate	47 (26.9)		15 (8.6)	
Occupation				
Employee	72 (30.1)	0.52	15 (6.4)	0.75
Student	21 (17.2)		9 (7.4)	
Housewife	10 (11.2)		7 (7.9)	
Farmer	8 (32)		5 (20)	
Others	5 (29.4)		1 (5.9)	

Chi-square test was used

* Statistically significant (p-value < 0.05)

Table 3: Frequency of signs and symptoms among patients

Signs and Symptoms	n (%)
Fever	489 (99.7)
Headache	408 (83.3)
Body ache	356 (72.7)
Retro-orbital pain	336 (68.5)
Nausea	301 (61.4)
Vomiting	260 (53.1)
Bleeding	200 (40.8)
Diarrhea	196 (40)
Abdominal pain	78 (15.9)

Table 4: Complete blood count in dengue patients

Complete Blood Count	Mean ± S.D
Hb (g/ dl)	11.0±1.8*
MCV (µm ³)	74.8±8.4*
MCH (pg)	26.6±2.9
MCHC (g/ dl)	31.3±2.9
RBC (m/ cm)	4.3±0.7
Leucocytes	3 × 10 ⁹ /L ±11.4
Platelets (m/ cm)	114 × 10 ⁹ /L*
Basophils (%)	ND
Eosinophil (%)	2.1±0.6

Lymphocytes (%)	3.2±7.3*
Monocytes (%)	2.0±1.1*
Neutrophil (%)	65.8±10.2*

*Hb, Hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration.

Discussion

DENV poses a significant pandemic threat in numerous regions worldwide. The dengue virus, belonging to the Flaviviridae family, exists in four closely related serotypes (DENV-1, DENV-2, DENV-3, and DENV-4). Once infected, humans serve as the primary source and amplifiers of the virus, creating a reservoir for uninfected mosquitoes. The main method for preventing the spread of DENV is through the control of mosquito populations. Over the past ten years, several outbreaks have occurred in the country, resulting in thousands of infections and some fatalities (7).

This study assessed the clinical manifestation and changes in hematological profiles of dengue patients. The disease predominantly affected individuals aged 11–50 as compared to the elders. Because higher number of participants had more outdoor exposure as compared to older. Other studies on dengue patients also supported that the intensity of dengue was high in young age group as compared to the old age group (11). In our study the results were same as the higher number of patients were presented in age category 16–35 years.

Male participants were more commonly affected as compared to females, possibly due to occupational exposure and outdoor activity and had more chances for the dengue (12). Our results showed the same results as male were almost 57%.

In our study, 67.2% cases were urban and 32.8% were rural. In the recent study, among 175 confirmed cases, 103 (58.9%) were urban compared to 74 (41.1%) were from rural area, reflecting dengue's urban dominance (13). Nonetheless, contradictions have surfaced as well. Various elements could lead to a higher prevalence in urban areas, such as increased population density in cities, inadequate sanitation systems, the prevalence of artificial water containers that create breeding grounds for vectors, and heightened human mobility and interactions in urban settings (14).

The DENV IgG seroprevalence (22.9%) aligns with findings, (21.8%) and (24%). (15) However, some findings were opposite (56.8%) and (33.3%) (16), and higher than in Tanzania (7.7%) and Kenya (12.5%) (17).

The IgM prevalence was 7.6% in our study. The rate of anti-DENV IgM seropositivity was 7.9%, suggesting recent DENV infection. IgM for the DENV infection can typically be identified approximately 5–7 days after onset. Nevertheless, it is important to note that IgM

antibodies might initially be negative for a few days, and the reactivity of IgM is non-specific; consequently, cross-reactivity may occur due to infection with other flaviviruses (5).

Prior reports indicated that the seropositivity rate for DENV was greater in centrally located areas (urban centers) compared to rural villages. A recent mosquito bite was found to have a significant correlation with anti-DENV IgG seropositivity. This aligns with the understanding that mosquito bites expose people to dengue fever (DF), which may be the primary mode of transmission in the area examined. Nevertheless, factors such as age, location of the study, and occupation showed minimal impact on the rate of exposure to DENV in the current research (18) and these results were correlated with our study.

The main variant of the dengue virus found in Pakistan is recognized as DENV-2, according to research results. Nonetheless, it is important to note that the occurrence of the virus or its variants may change over time and vary among different regions in the country. Additionally, a study conducted at the National Institutes of Health (NIH) in Islamabad examined 343 human serum samples in 2022, all of which were confirmed positive for the dengue virus NS1 (19).

These inconsistencies can be attributed to several factors, including when the sample is collected, variations in testing methods, the diversity of patient demographics, and specific dengue serotypes present. The small proportion of cases (6.85%) that demonstrate both NS1 and IgM positivity is likely a result of the limited period during which both markers can be detected at the same time (20). Our study showed the significant changes in CBC report of all the participants. A recent study showed that the CBC results revealed significant changes in neutrophils, leukocytes, monocytes, and platelets, aligning with

dengue-induced hematological alterations like leukopenia, lymphopenia, and thrombocytopenia, which can signal disease severity (4).

Hematological issues characterized by low platelet counts were observed in 90% of the patients. These results align with previous reports of 80% and 92% (14). A different team of researchers from India linked thrombocytopenia, leucopenia, and hemocentration to dengue infection (21).

Using symptoms as the basis for our assessment, we checked the clinical features of dengue-positive cases. According to the results, which included a priori confounders and adjusted for treatment type, elevated body temperature, and days before fever onset, indicated that individuals with dengue were more likely to experience retro-orbital and abdominal discomfort. Retro-orbital pain is a well-known symptom of dengue and is included in the 1997 case definition that distinguishes between probable and confirmed dengue fever (DF) as well as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Abdominal pain, recognized as a common gastrointestinal symptom of dengue, has also been correlated with more severe and hospitalized cases of the illness (22).

Fever, headache, body ache and retro-orbital pain were the most common symptoms, consistent with studies. Severe signs like persistent vomiting, bleeding, and abdominal pain were linked to advanced disease stages (23). The most common symptoms were headache, fever, retro-orbital pain, nausea and vomiting were shown in our results.

The notable relationships among symptoms such as headaches, chest pain, and gastrointestinal issues are significant. These findings align with a study that identified strong correlations between headache, chest

discomfort, abdominal pain, and the severity of dengue, yet contrast with another research (22). This discrepancy might arise from differences in sample sizes, research methodologies, regional variations in how dengue manifests, the timing of symptom evaluations, the most common serotypes or strains of the virus, access to healthcare, treatment guidelines, and the prevalence of comorbidities or confounding factors within the study populations.

Limitations

The main limitation of this study was short time period and sample size. Study should be conducted over a longer period of time to assess seasonal variation and other risk factors for the dengue fever. Further research is needed to evaluate climatic influences and enhance intervention strategies.

Conclusion

This study demonstrates a substantial burden of dengue infection, with IgG seropositivity exceeding IgM positivity and significant associations with sex and place of residence. The predominance of headache, body ache, retro-orbital pain, and characteristic hematological abnormalities highlights the importance of early serological and clinical assessment for timely diagnosis and management. These findings support strengthened dengue surveillance and targeted preventive strategies, particularly among populations at increased risk.

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