Original article

Dengue Fever: Prevalence, Risk Factors and Serological Diagnosis in Hyderabad, Sindh, Pakistan

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ABSTRACT

Introduction: Dengue viral infection (DVI), transmitted by mosquitoes, is a significant global health concern, especially due to climate change in tropical and subtropical areas. It manifests in severe forms like dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), affecting vital organs. In Pakistan, dengue is endemic with seasonal outbreaks, particularly after monsoons, affecting younger populations more severely. The study investigates the prevalence, risk factors, and serological diagnosis of DVI in Hyderabad, Sindh, Pakistan. The current study was designed to investigate the serology, prevalence, risk factors, mortality and morbidity of dengue fever in Hyderabad, Sindh, Pakistan.

Subjects and methods: A case-control study was carried out from 2010 to 2017 at the Civil Hospital of Liaquat University of Medical and Health Sciences, Hyderabad, Sindh, Pakistan in collaboration with the University of Sindh, Jamshoro. Blood samples from 18,320 cases aged 13-75 years, suspected of dengue were analyzed. A questionnaire assessed risk factors, and serology was evaluated through rapid strip tests detecting dengue antigens and antibodies (IgA, IgM, IgG). Statistical analysis, including logistic regression and odds ratio calculations, was performed using SPSS.

Results: A total of 18,320 suspected cases were subjected to the strip test for dengue fever. Among these, 1312 (7.2%) cases were found serologically positive. Dengue fever severely affected the population in during the study duration. A higher percentage of dengue-specific IgM-positive (59.6%) cases indicate the primary infection, while a lower percentage of patients with positive IgM and IgG (24.6%) results specify the secondary infection. Result showed the higher prevalence in males patients compare to females, majorly involving dengue-fever, -hemorrhagic fever and-shock syndrome. Furthermore, significantly ($p<0.05$) reduced levels of leucocytes, monocytes, neutrophils, and platelets were observed in dengue patients than in control cases.

Conclusion: Dengue is a serious endemic infection in Hyderabad, with primary infections being more common. Effective preventive measures, including public awareness and the use of mosquito repellents, screen doors, and covered water containers, are crucial to reducing transmission. Further research is needed to explore climatic factors and enhance preventive strategies.

Keywords:

Serological, Pakistan, Epidemiology, Prevalence, Risk factors, Dengue fever.

INTRODUCTION

Dengue viral infection (DVI), transmitted by mosquitoes, has become an increasing health concern to climate change in tropical and subtropical areas. It belongs to the Flaviviridae family and is classified as an RNA virus, with four genetically distinct serotypes: DENV-1 to DENV-4. Clinical manifestations vary, with severe cases leading to dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), which can impact the heart, liver, brain, and other organs[1-3].

Over 2.5 billion people are at risk of DVI, as it is prevalent in over 100 countries[4].The World Health Organization (WHO) estimates that there are 50-100 million annual cases of DVI globally, resulting in 250,000-500,000 cases of DHF and about 24,000 deaths[5, 6].

Factors contributing to the global rise in dengue include unplanned urbanization, climatic and socio-economic factors, seasonal influences, and an increase in waste containers. Multiple dengue serotypes are circulating simultaneously, a condition known as hyperendemicity. Other contributing factors include the increased use of disposable items that serve as mosquito breeding grounds, weakening public health systems, insecticide resistance, and climate change[7-8]. The recent rise in dengue cases has been linked to poor infrastructure, demographic changes, and inadequate environmental sanitation[4]. Water storage containers and solid waste provide ideal breeding sites for mosquitoes, especially during the rainy season in areas lacking piped

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water. Community behaviours can either increase or decrease the risk of the disease. Increased travel and transportation, along with socio-demographic and ecological factors, have facilitated the spread of dengue to new areas[5].

The first reported outbreak of dengue fever in Pakistan occurred in 1982, with various serotypes involved[9]. DHF cases have been sporadically reported across the country. In 2006, DV-2 and DV-3 were identified as the most severe and prevalent serotypes in Pakistan. Demographic differences in dengue and DHF cases have been observed, with multiple serotypes found in Lahore during the 2008 outbreak, leading to a higher prevalence of DHF[10-12]. DVI is now endemic in all provinces of Pakistan, with cases reported throughout the year, particularly after the monsoon. Younger populations are more susceptible[13]. Recent floods in Sindh, Pakistan have likely contributed to the spread of the disease by altering risk factors[14]. Dengue epidemics also create hyperendemic, typically large and dense populated areas. The available literature is limited to the prevalence serotypes. No study has been carried out at Hyderabad related to serology, risk or protective factors, or climatic association. The current study aimed to explore the climatic and biochemical associations and identify risk factors associated with dengue outbreaks in the Hyderabad a population based case-control study.

SUBJECTS AND METHODS

The review board committee Institute of the Biochemistry University of Sindh, Jamshoro, approved the project with reference no. 317/2022. The specimens were collected from dengue patients and controls after written informed consent.

This case-control study was carried out at Liaquat University of Medical and Health Sciences Hospital, Hyderabad, in collaboration with the University of Sindh, Jamshoro. It included both inpatients and outpatients, aged 13 to 75 years, suspected of having dengue from 2010 to 2017. A total of 18,320 blood samples were collected from patients experiencing fever along with other clinical signs and symptoms of DVI. Dengue diagnosis was based on the international/national guidelines of Centers for Disease Control (CDC) and Dengue Expert Advisory Group (DEAG) provided about laboratory confirmatory tests include serology dengue virus protein (NS1 antigen) antibody detection[15]. All samples were tested for malaria, and malaria-negative patients, along with sixty geographically and gender-matched controls, were included in the study.

A specially designed standard questionnaire was used in this study to investigate the epidemiology of DVI in relation to prevalence, gender, and various environmental factors. Risk factors were including covered water containers, water containers without cover, flowerpots, placed inside and spare tyres outside the house. Comprehensive medical histories were obtained, and blood samples were collected from all participants.

Approximately 3mL intravenous blood samples were collected in an EDTA tube for the analysis. All the samples were checked for the presence of malarial parasites by creating a peripheral smear of patient blood with Giemsa stain. The results were confirmed under a microscopic examination at a 1000-fold magnification. The serology of dengue was examined by the rapid strip test (Pan Bio, Australia). Sensitivity of Pan Bio kit for the dengue detection is 91.89%, and its specificity is 98.39% where as its correct identification for dengue in blood samples has been reported by Hussain et al., 2022[16]. To detects dengue antibodies for the presence of NS1 antigen, immunoglobulin A (IgA), immunoglobulin M (IgM) and immunoglobulin G (IgG). The sensitivity of antibodies, antigen for the diagnosis of dengue has reported for IgM and IgG 90%, NS1 100% and IgA high sensitivity and specificity in the acute phase dengue. The occurrence of only anti-dengue IgM antibodies indicated primary infection. Both IgM and IgG positivity indicated secondary infection in a smaller population as compared to primary infection[17]. DVI patients having any bacterial, parasite, or viral infection positive were excluded from the study. Signs and symptoms of dengue include high-grade fever, headache, vomiting, transient macular rash, nausea, abdominal pain, diarrhea, and hemorrhagic manifestations. Leucopenia is a predictor of dengue and thrombocytopenia for DHF; a complete blood count was determined to observe the recovery trends of dengue patients[18].CBC after the confirmation of DVI was analyzed by a Sysmex KX-21 haematology analyzer.

Percentages and frequencies were calculated for qualitative variables, whereas means and standard deviations were calculated for quantitative variables (complete blood count parameters). To determine the association between qualitative variables and dengue infection, odds ratios (OR) with 95% confidence intervals (CI) were calculated to assess the association between each qualitative variable (including covered water containers, water containers without cover, flowerpots, spare tires, screen doors, and windows) using the Chi-squared test. SPSS software was used for data analysis, and a p-value less than 0.05 was considered statistically significant.

RESULTS

Overall, 1312 (7.2%) patients were found positive for DVI from 18320 suspected cases and among positive dengue

cases seven patients (0.5%) were died. The present study reported the several risk factors of dengue such as covered water containers (36.6), uncovered water containers (40%), flowerpots (66.6%) inside and spare tyres (66.6%) were placed outside the house associated with dengue fever **(Table 1)**. A significant association was seen with uncovered containers, flowerpots, and spare tyres indicated by odds ratios of 2.9, 2.8, and 7.2 respectively. Results of preventative measures included use of mosquito spray (30%), coil repellant (46.6), sleeping net (16.6%) or doors (66.6%) and windows (73.3%) with screens were inversely related to DVI with odds ratio ≤0.8 **(Table 1)**.

Serology of DVI

Among all the positive dengue cases, 78 (5.9%) tested positive for the NS1 antigen. Serologically, 83 (6.3%), 323 (24.6%), and 782 (59.6%) patients tested positive for IgA, IgG, and IgM antibodies, respectively. The presence of IgA, IgM, and IgG were linked to early, primary and suspected secondary DVI. The study indicated that primary infection affected a larger population (59.6%), while a smaller number of cases were identified as secondary infections (24.6%) **(Table 2)**

Clinical presentation

Table 3 compares the signs and symptoms of dengue-positive patients and controls. The most common symptom among dengue patients was high-grade fever, reported in (99.9%) cases. Other prominent symptoms included nausea (44.2%), headache (83.4%), body discomfort (25.9%), and vomiting (19.8%). Severe cases presented with bleeding from the rectum (1.1%), bleeding in urine (45.4%) gums (1.4%), mouth (3.4%), and nose (2.2%). A comparison of CBC counts between dengue patients and controls showed significant reductions ($P<0.05$) in neutrophils, platelets, leukocytes, and monocytes **(Table 4).** Analysis of clinical classifications revealed that the severe stage of dengue fever was significantly less associated with dengue shock syndrome (DSS) compared to DHF **(Figure 1).** Dengue Hemorrhagic Fever (DHF) involves increased capillary permeability, leading to plasma leakage and bleeding, while DSS is a more severe progression of DHF, characterized by circulatory failure and hypovolemic shock due to critical fluid loss.

Year-wise incidence of disease

The occurrence of dengue cases during 2010-2017 is shown in **Figure 2.** This emphasizes the large dengue outbreaks that affected a large population in 2010, 2013, and 2016. Additionally, the frequency of male patients steadily stays higher than that of female patients through the entire study period.

Month-wise incidence of disease

The study found that dengue virus infection (DVI) in the area was significantly influenced by weather conditions. Based on seasonal patterns, the area was categorized into three periods: post-monsoon (October-January), early rainy season (February-May), and monsoon (June-September). Most cases were recorded during the post-monsoon season, particularly in October and November (Figure 3).

Table 1: Predisposing and protective factors associated with dengue: comparison between cases and controls

Characteristics	Controls, %	Cases, %	Odds ratio	p-value
Risk Factors				
Empty house	25	26.6	1	0.8
Spared tyres	21.6	66.6	7.2	0.0001
Covered water container	53.3	36.6	1.5	0.4
Uncovered water container	18.3	40	2.9	0.02
Flower pots & backyard planting	41	66.6	2.8	0.04
Protective factors				
Screen doors	80	66.6	0.5	0.1
Screen windows	95	73.3	0.1	0.003
Mosquito coil	51.6	46.6	0.8	0.6
Insecticide spray	45	30	0.5	0.1
Sleeping net	25	16.6	0.6	0.3

Table 2: Serology distribution of dengue patients was during the outbreak of 2010 to 2017

Years	Serology (n= 1312)				
	NS 1 antigen	IgM	IgM + IgG	IgG	IgA
2010	38	278	96	11	9
2011	0	42	32	2	21
2012	0	4	0	0	0
2013	40	248	145	21	43
2014	0	1	0	0	0
2015	0	40	5	2	4
2016	0	143	45	10	6
2017	0	26	0	0	0
Total	78 (5.9%)	782 (59.6%)	323 (24.6%)	46 (3.5%)	83 (6.3%)

Table 3: Frequency of Sign and symptom from the year 2010 to 2017

Signs and Symptom	No. Patients n= 1312	Percentage
Fever	1311	99.9
Headache	1095	83.4
Nausea	580	44.2
Body Ache	340	25.9
Vomiting	260	19.8
Bleeding	198	15.1
Diarrhea	80	6.1
Abdominal Pain	68	5.2
Bleeding from Mouth	45	3.4
Bleeding from Nose	29	2.2
Bleeding from Gums	19	1.4
Bleeding from Rectum	15	1.1
Bleeding in urine	90	45.4

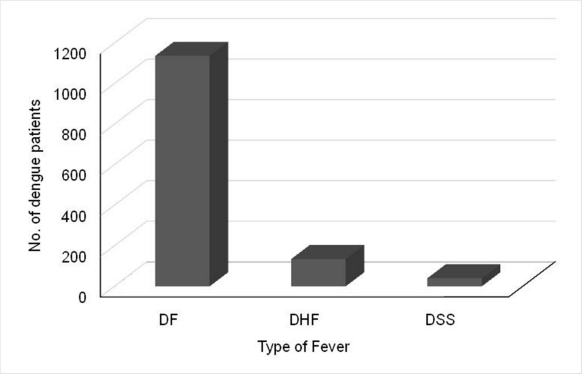

Figure 1: Clinical classification of dengue fever during outbreak

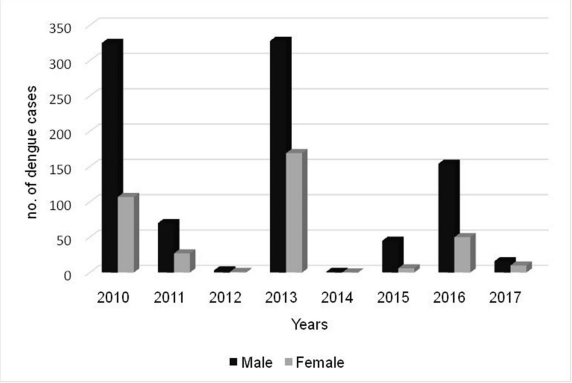

Figure 2: Dengue case compared from 2010 to 2017 in Hyderabad, Sindh, Pakistan.

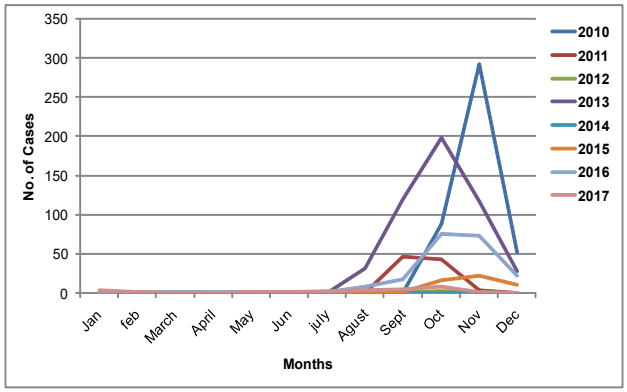

Figure 3: month wise comparison of dengue patients during outbreak.

Table 4: Comparison of complete blood count between dengue patients and controls

Complete Blood Count (Mean ± SD)	Cases	Controls
Hb (g/dl)	11.0±1.8*	13.2±0.*
MCV (µm3)	74.8±8.4*	92 ±6.3*
MCH (pg)	26.6±2.9	31 ±2.4
MCHC (g/dl)	31.3±2.9	33±2.4
RBC (m/cmm)	4.3±0.7	4. 8±0.5
Leucocytes	3 x 109/L ±11.4*	7 x 109/L ±17.0*
Platelets (m/cmm)	114 x 109/L*±509	300 x 109/L* ±102
Basophils (%)	ND	0.5 ±0.5
Eosinophil (%)	2.1±0.6	2. 0±0.6
Lymphocytes (%)	3.2±7.3*	4.4 ±1.3*
Monocytes (%)	2.0±1.1*	31.7 ±5.4*
Neutrophil (%)	65.8±10.2*	59 ±4.4*

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

DISCUSSION

This study showed the seasonal incidence of dengue occurrence is connected with their breeding sites and population abundance. The current study revealed risk factors such as covered water containers, water accumulated in sinkholes, freshwater containers without cover, flowerpots inside the house, and spare tyres. Among them, three key risk factors that were positively associated with the spread of dengue fever were spare tires, indoor water storage containers without covers, and water accumulating in sinkholes. Current study results of preventative measures included that the used of pesticide spray, mosquito repellant, or doors, and windows with screens were inversely related to DVI. Screen windows were a significant ($p<0.003$) protective factor from dengue. Various risk factors including, lack of a community potable water supply, wooden housing, vacant homes or land plots in neighborhoods, lower socioeconomic communities, and unplanned gardening are related to the spread of dengue disease studies reported the impact. One of the previous of these factors in dengue affected the populations[19].

Different studies reported that dengue was predisposed by the assessment of location for the investigation, exposed and circulating virus serotype strain, human population density, and humidity, obtained ability of oviposition sites, and previous ambient temperature[20-22]. DVI is greatly influenced by the weather in the study area. Keeping in view of the seasonal settings, the area was divided into three classes which comprise post-monsoon (October-January), the early rainy season (February-May) and monsoon (June-September). The majority of cases were documented in the post-monsoon season. These months' temperatures were ideal for altering the population dynamics of Aedes mosquitoes and their ability to reproduce. Hyderabad has an arid climate with a lower basin that receives mean annual rainfall of less than 200 mm, and annual mean temperature ranges between 17 and 28 ◦C. Whereas the

temperature from the October to November was approximately 28 to 20°C which is suitable for mosquitoesfertilization[23] temperature plays an essential role in influencing the population dynamics of Aedes mosquitoes and minimum temperature was positively associated with dengue cases when the temperature was less than 21.3 ◦C[24]. According to Ramachandran et al.[25], regular heavy rainfall in India caused a decrease in temperature throughout the last session of the monsoon season. The temperatures during these months, coupled with high humidity, created ideal conditions for Aedes mosquito breeding, larval growth, and virus transmission. Due to heavy rains and unanticipated flooding in Sindh from July to September, dengue incidence increased in 2010, 2013, and 2016. August saw the greatest effects of the floods, which moved south along the Indus River and produced perfect breeding circumstances for mosquitoes, including high humidity, freshwater reservoirs, and excellent temperatures. According to earlier research, men were more impacted than women, maybe as a result of increased outside exposure[26-28].

The serological prevalence study populace was infected with dengue fever among them primary infection was shown by a greater proportion of dengue-specific IgM-positive antibodies. Both IgM and IgG positivity indicated secondary infection in a smaller population as compared to primary infection. IgG positivity alone was detected in fewer cases revealing the suspected secondary infection. The occurrence of only anti-dengue IgM antibodies indicated primary infection. However, the presence of anti-dengue IgG antibodies with or without IgM indicated secondary infection. Only IgG antibodies presence was considered a suspected secondary infection as it could also be due to cross-reactivity with other flaviviruses. Dengue patients' memory B cells rapidly secrete significant levels of IgG, and various antibodies secreted in the acute stage of secondary DVI exhibit cross-reactivity for many serotypes[29].

Comparable signs and symptoms were reported in other epidemic areas like Southeast Asian countries. Although a higher number of patient's experience vomiting and abdominal pain, a non-specific symptom called a headache has also been recorded[30]. Clinical presentation of dengue fever in a current study demonstrated that the severe stage of fever was significantly lesser with DSS than with DHF. Individuals with higher or lower pre-existing antibody titers showed no increased risk of DHF or DSS. Only when pre-existing antibody titers are within a limited intermediate range is the risk of severe dengue increased. Viral replication is activated by intracellular antiviral innate immune pathways, which may contribute release of potentially harmful inflammatory products[31]. Results of CBC count were compared to dengue patients and controls interpreted that neutrophils, platelets, leucocytes and monocytes significantly (P< 0.05) reduced in patients. Since dengue fever has been associated with morphological and functional changes in blood cells prognostic for dengue. Leucopenia, lymphopenia, and thrombocytopenia of CBC alterations may offer early indicators of the severity of the disease[18].

CONCLUSION

The study concludes that dengue is a significant endemic infection in Hyderabad, with primary infections being more common than secondary ones. The transmission is influenced by climatic factors, particularly the post-monsoon season, which creates ideal conditions for Aedes mosquito breeding. Key risk factors include uncovered water storage, stagnant water accumulation, and inadequate preventive measures. Effective control strategies, such as public awareness, the use of mosquito repellents, screened windows, and covered water containers, are crucial to reducing dengue transmission. Further research is needed to understand climatic factors and improve preventive efforts.

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