

## ORIGINAL ARTICLE

# THE CLINICAL MANIFESTATION AND LABORATORY PARAMETERS OF DENGUE NS1 ANTIGEN POSITIVE PATIENTS IN HYDERABAD, SINDH

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## ABSTRACT

**Background:** Dengue is a viral infection caused by the dengue virus, which is transmitted to humans through the bite of infected mosquitoes. This analysis was carried out to assess the occurrence of Dengue Fever in the district of Hyderabad to evaluate the demographic, clinical and haematological characteristics.

**Materials & Methods:** From August 2024 to December 2024, the randomized, prospective research was conducted on 120 patients, suffering from dengue fever, confirmed by NS1 antigen positive. The data was collected by the directly questioning method by well-designed questionnaire at civil hospital (Hyderabad, Sindh). Complete blood counts (CBC) were also done. A detailed clinical history was taken.

**Results:** Out of total 120 cases of dengue, 72 cases (60%) were males and 48 (40%) were females. According to age, maximum cases 42 (36%) were in 21-30 years, represents the highest prevalent group. The average haemoglobin level is mean 12.14 g/dL. The difference between haemoglobin was very large and highly significant ( $t = 69.64$ ,  $df = 119$ ,  $p = <0.001$ ). The higher section of the patients represents thrombocytopenia whereas the smaller section illustrates that only 25.8% have normal platelet counts, the statistical evidence of difference was the strongest with haematocrit ( $t = 91.91$ ,  $df = 119$ ,  $p = <0.001$ ). The red blood cell (RBC) count remains relatively stable with a mean of 4.39 million/ $\mu$ L, while white blood cell (WBC) levels average  $4.29 \times 10^3/\mu$ L, indicating a trend toward leukopenia.

**Conclusion:** Our analysis indicated that CBC is the most crucial identification for dengue as it reveals warning signs like low platelets and low white blood cells. We also conclude that proper surveillance of dengue is essential whereas awareness campaign must be expanded upto the larger population.

**KEY WORDS:** Dengue fever; Leukopenia, NS1 Antigen; Thrombocytopenia.

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## INTRODUCTION

Dengue is an emerging mosquito borne infection.<sup>1</sup> It has been a major health concern for decades. The first case of dengue fever in Pakistan was recorded in November 1994 since then Pakistan has seen multiple recurring dengue epidemics in different regions. The

disease is commonly called as “Break Bone Fever” or “Bone Crush Disease” because of the disease’s main side effects, which include myalgias (pain in muscle) and arthralgias (pain in joint).<sup>2</sup> Currently, it is projected that 50% of the world’s populace lives in areas where they are at risk of contracting dengue arboviral disease, and there are about 100 million dengue cases reported annually worldwide. Dengue is a mosquito-borne viral infection of the twenty-first century that is considered a threat to public health and causes significant socioeconomic burden on many tropical, subtropical, and temperate regions of the world.<sup>3</sup> The WHO estimates that two-thirds of the world’s population, especially those in tropical and subtropical nations, are at risk of contracting dengue virus infection. Although dengue is prevalent in many nations, Southeast Asia, and the western

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Pacific account for most occurrences.<sup>4-6</sup>

Prior to the 18th century, when the first recorded epidemic of dengue-like illness started with severe consequences, the comparable ailment was widely distributed geographically. Dengue is the primary cause of individuals getting hospitalized.<sup>7</sup> The illness is currently the fastest-growing virus spread by mosquitoes and is widespread in about 129 countries. The number of dengue cases reported in some parts of Pakistan has increased tenfold in the past fifteen years. The unusually heavy monsoon rains in 2022 caused the worst flooding Pakistan has seen in recent memory. According to historical statistics, there is a considerable correlation between the amount of rainfall and rises in dengue incidence. Thus, the nation has experienced severe disaster because of the recent severe flooding and the massive increase in dengue cases.<sup>8</sup> An estimated 25,000 deaths occur worldwide each year. In 1994, the first dengue fever outbreak was recorded in Pakistan. Dengue epidemics in several districts of Punjab province, including Lahore, Gujranwala, Sheikhpura, Faisalabad, Rawalpindi and Attock occurred in 2010–2011. These were followed by outbreaks in Swat and Mansehra in 2013–2014.<sup>9</sup> The World Health Organization (WHO) estimates that 390 million cases may occur annually, with Asia bearing a 70% burden. As of November 25, 2021, the nation had recorded 48,906 dengue cases, including 183 fatalities; Punjab had the most reports.<sup>10</sup> One significant viral infection that is transmitted by arthropods is dengue virus (DENV) infection. The four antigenically related but different serotypes of the dengue virus, which belongs to the Flaviviridae family, are DENV 1, DENV 2, DENV 3, and DENV 4. Between primary and secondary infections, as well as potentially between various dengue virus serotypes, the disease's incidence and severity vary.<sup>11</sup> The newest and fifth addition to the prevailing serotypes of dengue viruses is DENV 5 which has been proclaimed in October 2013.<sup>12</sup> It is spread by *Aedes* mosquitoes, mainly *Aedes aegypti*, which are members of the *Aedes* family. However, other *Aedes* mosquitoes, such as *Aedes polynesiensis*, *Aedes albopictus* and *Aedes scutellaris*, have also been observed to serve as vectors. *Aedes aegypti* is a daylight feeder with topmost number of mosquito bites usually seen at early mornings and dusk.<sup>13</sup>

There were nine nationwide epidemics in between 1994 to 2013. Compared to other Asian locations, Lahore (2011) and Swat (2013) have extremely high reported dengue cases and mortality rates. The country had unusual weather conditions in 2010, including flooding and strong rainfall, based on the climatic data. Dengue may have increased because of floodwater accumulation in the nation's low-lying areas and a large influx of individuals from nearby cities seeking work and education.<sup>14</sup> The clinically

dengue ranges from an asymptomatic infection to an identical fever to 3 conditions dengue fever (DF) and Dengue haemorrhagic fever (DHF) and DHF further can lead to Dengue shock syndrome (DSS). A patient's complete blood count (CBC) is a crucial component of their diagnostic process.<sup>15</sup> Incubation usually lasts three to fourteen days, and the sickness can progress from asymptomatic to potentially fatal. Fever, critical, and recovery are the three stages of an infection. Fever usually lasts two to seven days and is abrupt in onset. Muscle and joint pain, nausea, vomiting, rash, diarrhoea, stomach pain and leucopenia may accompany it. Vein leakage can result in thrombocytopenia.<sup>16</sup> High temperature, muscular and skeletal pain, strong headaches, and rash are clinical signs of dengue fever. Dengue Haemorrhagic Fever (DHF) is characterized by the appearance of rash or haemorrhagic symptoms in addition to the traditional Dengue Fever (4). Thrombocytopenia and concentration of blood are common findings. From June 1994 to September 1995, Karachi, Pakistan, experienced a widespread dengue virus outbreak. In terms of morbidity and mortality, dengue is the most significant arbovirus disease affecting humans.<sup>17</sup>

The specific dengue test, which is frequently used for early dengue diagnosis with notable sensitivity and specificity, is the dengue viral non-structural protein (NS1) antigen test. The glycoprotein known as dengue NS1 antigen, which is generated in both secretory and membrane-associated forms, is found in high concentrations in the blood of people who have contracted dengue during the early stages of fever. With great sensitivity, it could be seen from day 1 to day 5 and, in certain situations, up to 9 days after the illness started. Additionally, it can be found in dengue infections that are primary or secondary. NS1 testing can be carried out in a hospital without laboratory space, even though other dengue confirming tests need a well-equipped lab.<sup>18</sup> Current study based on Clinical Manifestation and Laboratory Parameters of Dengue NS1 Antigen Positive Patients in Hyderabad. This study was specifically designed to assess the clinical and blood parameters in Hyderabad, Sindh. The target of this research is to analyse the patients and fluctuations in different features, it will help to recognize the severity of disease in forthcoming time.

## **MATERIALS AND METHODS**

A randomized, prospective study was conducted in the Hyderabad, Sindh a province of Pakistan. The district is the second most urbanized city in Sindh. The data of patients was collected from Civil Hospital Hyderabad from August to December 2024 during this interval 120 cases of Dengue fever were included in the study. Dengue NS1 antigen test was done to check the antigen status of the patients. This test allows rapid detection on the first day of

fever before the appearance of antibodies (IgM). All the patients were confirmed as having dengue fever by Dengue NS1 Antigen positive test. Dengue NS1 Antigen is a non-structural viral protein and is a marker for early protein (1-9) days. A specially designed Performa was used for the data collection. Proforma used in this study was based on three sections and categorized on WHO standard. The first section consists of demographic variables like sex, age. Second section comprise of clinical signs and symptoms. Third section included the haematological outcome like haemoglobin, platelets count, red blood cell, white blood cell. No patient in our study population was expired.

An institutional verbal consent was obtained by The Head of the institute. Verbal consent was taken from every patient hence the name and other information of patient is not revealed. All the data were collected from patients and from hospital will be exclusively used for the research purpose only. In this research only dengue fever cases were included however DHF and DSS patients were excluded. In dengue fever cases only NS1 antigen test patients were included as it detects in early days and study focus on milder cases not in severe cases of dengue fever. The data included in this only comprises demographic, clinical and laboratory profile. Patients were excluded who doesn't have CBC report for more confirmations. This research consists of the initial CBC report only before any follow-up.

Data Analysis: Every section of the questionnaire was analyzed and tabulated for its better understanding by using SPSS-27. Before the entry of data on SPSS all the Performa were examined of missing information. Firstly, demographic data was tabulated age wise, and sex wise secondly clinical data was summarized in the last frequency of hemoglobin, thrombocytopenia and leucopenia was evaluated. Data was examined for the frequency, mean and standard deviation for different variables. Haematological parameters were compared by T-Test and ANOVA.

**RESULTS**

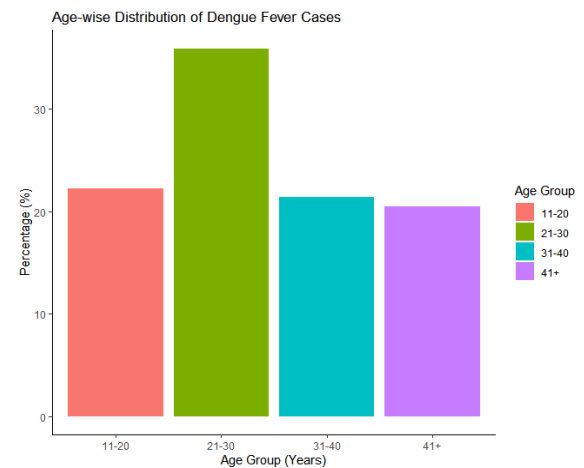
The 120 cases who were investigated in this study are given in table 1, patients were both males and females, 72 cases (60%) were males and 48 (40%) were females. The incidence of dengue fever was detected more in males than females. The highest case in males indicates the greatest exposure of dengue mosquito in environment. We looked at dengue fever prevalence by age. The patients were divided into four groups: those aged 11–20, those aged 21–30, and those aged 31-40 and last one 41-up. The most impacted age group with the highest prevalence (36%) was found to be the age group (21–30 years). In addition, this research reveals that dengue fever mainly affects 11 to 40 years as

shown in Figure 1.

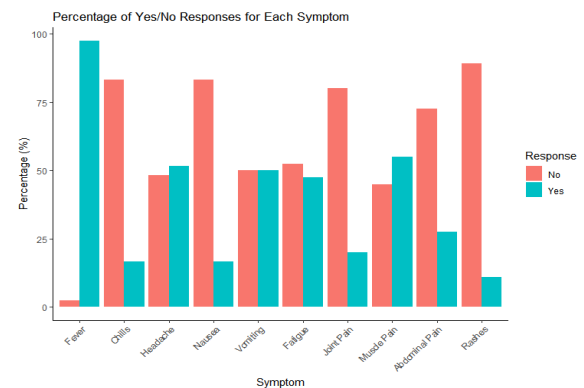
Clinical features of dengue fever cases are presented in Figure 2. Most common symptoms among the infected patients were noticed fever (97.5%), headache (52%), Myalgia or muscle pain (55%) and headache in (52%). Fever is the most dominant symptom confirming its status as a hallmark feature of Dengue infection showing in figure 2. Further frequently reported signs and symptoms involve vomiting (50%), fatigue (47.5%) and abdominal pain (27.5%) were also prominent but less prevailing.

**Table 1: Distribution of study population**

Sex	Count	Percentage
Female	48	40.0
Male	72	60.0
Total	120	100



**Figure 1: Distribution of dengue fever cases age-wise.**



**Figure 2: Clinical features of patients with dengue fever**

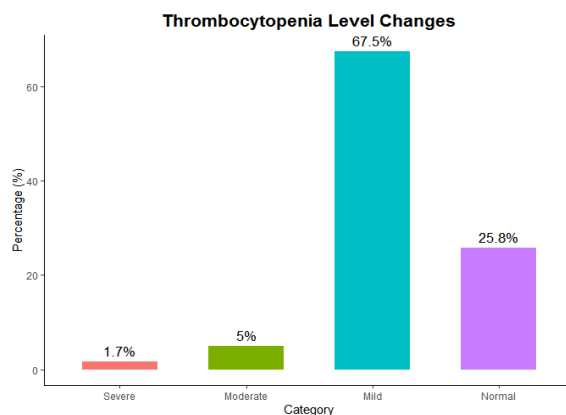
**Table 2: Alterations in haematological parameters in dengue fever positive cases**

Parameter	Mean	SD	Min	Max
Haemoglobin	12.14	1.91	7	16
Platelets	124.82	70.08	11	360
RBCs	4.39	0.64	2.9	6
WBCs	4.29	1.83	1.2	9
Hematocrit	38.4	4.58	24	47

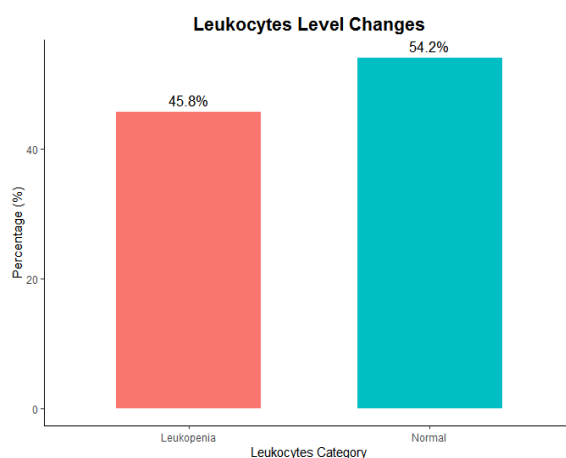
According to this investigation the haematological parameters shown in Table 2. The average haemoglobin level is 12.14 g/dL, with values limit from 7 – 16 this research suggests most of patients have normal level of while approximately some reported with mild anemia. Platelets count is markedly low with a mean of  $124.82 \times 10^3/\mu\text{L}$  indicating the main dengue fever complication as thrombocytopenia. RBCs were noted as normal in dengue fever however WBCs showed variations mean 4.29 and prove as most of the patients have leucopenia as a second important alteration in dengue fever patients.

Present study shows platelets count is a main trouble in dengue cases only 25.8% cases showed normal platelets while most of the cases 67.5% showed mild thrombocytopenia and 5% cases showed moderate thrombocytopenia along with this only (1.7%) cases indicated severe thrombocytopenia in Figure 3.

On the other hand, it showed that (45.8%) of the patients identified with leukopenia whereas (54.2%) had normal leucocytes Figure 4. This near equal distribution emphasizes that normal white blood cell is more common however low white blood cells also have significant proportion. It defines that nearly half of the dengue cases in Hyderabad have leucopenia.



**Figure 3: Thrombocytes changes in dengue fever**



**Figure 4: White blood cell levels in dengue fever positive cases**

The table 3 shows the results of t- test for all the haematological variables under examination. The difference between haemoglobin was very large and highly significant ( $t = 69.64$ ,  $df = 119$ ,  $p = <0.001$ ) and the mean difference of haemoglobin was 12.14 with a narrow 95% confidence interval (11.80 12.49)

**Table 3: T-test for haematological parameters in dengue fever positive cases.**

Variables	t	DF	P-value	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Haemoglobin	69.635	119	.000	12.14417	11.7988	12.4895
RBCs	74.620	119	.000	4.38842	4.2720	4.5049
WBCs	25.588	119	.000	4.28583	3.9542	4.6175
Platelets	19.509	119	.000	124.81667	112.1483	137.4850
Haematocrit	91.908	119	.000	38.40000	37.5727	39.2273

indicating a very precise and very strong estimate. Likewise, the count of red blood cell (RBC) showed a significant and statistically significant change ( $t = 74.62$ ,  $df = 119$ ,  $p = <0.001$ ), change in mean was 4.39 and the confidence interval is 4.27119. The count of white blood cell (WBC) also differed significantly ( $t = 25.59$ ,  $df = 119$ ,  $p = <0.001$ ), a difference in the mean of 4.29, and a difference in 3.95 to 4.62. Platelet counts were also significantly different ( $t = 19.51$ ,  $df = 119$ ,  $p = <0.001$ ), and the mean difference between the platelets was rather large (124.82), with a 95 per cent confidence interval between 112.15 and 137.49. Lastly, the statistical evidence of difference was the strongest with haematocrit ( $t = 91.91$ ,  $df = 119$ ,  $p = <0.001$ ), with the mean difference of 38.40 as well as a narrow confidence interval (37.57 to 39.23). In general, the  $t$ -values are very large, the  $p$ -values are extremely significant and the confidence intervals are very small which point to a high level of evidence of statistically significant difference and precision in the estimated effect of all variables.

## **DISCUSSION**

According to recent studies, dengue virus infection is prevalent in overall in the world, with the monsoon season seeing the highest rates. Three significant outbreaks with over 40,000 people nationwide have already been documented in 2006, 2010, and 2011. The outbreak of dengue virus waves could be the result of the current 30-fold rise in dengue virus cases from urban to rural and less affected areas. The next year may see a separate outbreak of these viral illnesses.<sup>19</sup> In our study we surveyed 120 dengue patients among which 60% were males and 40% were females represented in Table 1. Fouzia Khan et al conducted research on dengue fever cases in Karachi, Pakistan 60% were males and 40% were female's shows great sameness with our results.<sup>20</sup> Atul Garg et al from north India also analysed higher ratio in males than the females.<sup>21</sup> This highest prevalence in males than the females clearly indicates that male were more affected in the whole observed period. Moreover, Saara N et al collected the similar outcome from Kalaburagi, India with increased occurrence rate in males.<sup>15</sup> This research has also described that most of the cases were from age group (21-30 years) with the highest frequency. Anwar Faheem conducted research in KP, Pakistan, with the highest frequency (64.07%), the middle age group (19-37 years) was found to be the most afflicted, shows close similarity with our age groups.<sup>19</sup>

During the examination all the patients were observed for clinical symptoms of the disease which has been showed in Figure 2, fever was the most common symptom among all the patients in Hyderabad. In 2019, Jayadas et al. noticed common symptoms in dengue positive patients he identified fever,

headache and myalgia showed the closeness with our outcomes. All NS1 detected patients are included in both studies. Thrombocytopenia and leukopenia are well established features also shares great similarity.<sup>18</sup> Abid et al in district Shangla, KP, Pakistan observed dengue fever cases, we have similarity as in both research diagnosis is done by Non-structural protein-1 antigen (NS1) whereas males were more effected in both studies. In addition, clinical signs notified in our study are similar fever, body ache muscle ache headache and vomiting are common symptoms while problems such as bleeding and rashes were less dominant.<sup>19</sup> Haemoglobin level in dengue patients was not connected with the severity of the disease. Although there is no clear link between dengue infection and Haemoglobin, but research showed that Haemoglobin may also drop in some cases. Riffat Mehboob et al worked in Lahore, Pakistan and analysed most of dengue patients had normal Haemoglobin levels but it might show complex correlation with dengue patients as it may drop down in severe dengue fever cases.<sup>22</sup> Dengue patients typically have a platelet count of less than 150,000 per microliter, whereas the normal range is between 150,000 and 400,000 per microliter. Present study exposed 25.8% cases had normal platelets count while most of the cases 67.5% exhibited mild thrombocytopenia Another study was done by Irfan Arshad, in Karachi, Pakistan shown the most common hematological abnormalities were thrombocytopenia and leukopenia in dengue fever cases. Dengue fever has one strong hallmark and that is thrombocytopenia, the key reason for the low platelets count could be platelets destruction and bone marrow suspension. Moreover, monitoring to platelets count may help to assess the severity of dengue fever.<sup>23</sup> According to the observation of M. Hanif Ghani et al carried-out research in Hyderabad/ Jamshoro, Sindh (Pakistan) almost all cases developed mild to moderate thrombocytopenia shows great similarity with this study. Mild to moderate thrombocytopenia cases may recover from dengue fever. It prove that dengue virus inhibits bone marrow capability to produce sufficient platelets.<sup>24</sup> Surendra Nath Singh Yadav carried out research in 2018 on dengue patients and observed most significant laboratory abnormality was thrombocytopenia. Both studies share the common one abnormality in most of the positive cases that is platelets reduction. If severe low platelets count cases developed in dengue fever, it may lead to dangerous bleeding in this study only 1.7% cases suffered from severe thrombocytopenia.

## **CONCLUSION**

This study provided an update on the current prevalence of dengue fever outcomes in Hyderabad district. A total of 120 dengue-positive cases with NS1 identification had been identified in the present

investigation. It is concluded that dengue is more prevalent in male as compared to female. The most dominant age group among the suspected and confirmed dengue patients was 21-30 years, rather than in other groups. The most common hematological abnormalities were thrombocytopenia and leukopenia. However, in this study dengue infected patients mostly had mild thrombocytopenia. These clinical and blood cell parameters are not only helpful in evaluating the disease but also assist to understand the severity of the disease. There is a need for continuous surveillance and vector control activities as dengue outbreaks have become more common in Sindh in the past decade.

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**CONFLICT OF INTEREST**

Authors declare no conflict of interest.

**GRANT SUPPORT AND FINANCIAL DISCLOSURE**

None declared.

**AUTHORS' CONTRIBUTION**

The following authors have made substantial contributions to the manuscript as under:

Conception or Design:	ZC, NAB
Acquisition, Analysis or Interpretation of Data:	ZC, NAB, AH, LJ, NC
Manuscript Writing & Approval:	ZC, NAB, AH, LJ, FC

All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.



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