

Clinical and Biochemical Characteristics of Dengue Fever and Dengue Hemorrhagic Fever During the 2024 Dengue Outbreak

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Abstract

Background: Dengue is a vector-borne viral infection that poses a significant public health threat in tropical and subtropical regions, ranging from mild fever to severe forms like DHF and DSS. Gender and platelet count are thought to influence disease severity, but their roles remain unclear. **Objective:** This study aims to explore the demographic, clinical, and laboratory characteristics of dengue patients, focusing on gender differences and platelet count's association. **Methodology:** This was a prospective study carried out in the Department of Virology in NILMRC, Dhaka, Bangladesh from July to November 2024. The study population comprised confirmed dengue patients admitted to the Medicine Ward of AMZ Hospital during the study period, as well as outdoor patients from NILMRC, who met the inclusion and exclusion criteria. Serology and RT PCR were performed. **Results:** 57.9% were male and 42.1% were female, with a higher prevalence of dengue among males. The majority of patients were in the 16-25 (30.1%) and 26-35 (29.5%) age groups. Gender differences were observed in clinical features: males more frequently reported cough, headache, myalgia, fatigue, joint pain, and low platelet count, while females had a higher incidence of rash and nausea/vomiting. A higher proportion of males (8.2%) experienced severe complications like DHF/DSS. 84.2% having platelet counts $>100,000$ cells/ μ L. Elevated liver enzymes (AST and ALT) were observed in 42.5% and 37% of patients, respectively. DENV-2 was the predominant serotype (61%). Lower platelet counts ($\leq 100,000$ cells/ μ L) were associated with higher incidences of severe complications, elevated AST and ALT levels, and hematocrit rise $>20\%$. **Conclusion:** This study highlights significant gender differences in the clinical presentation and severity of dengue, with males more frequently experiencing musculoskeletal pain, fatigue, and severe complications like DHF and DSS. Platelet count emerged as a key marker for predicting severe outcomes.

Keywords: Dengue; DHF, DSS; Platelet count

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Introduction

Dengue is an acute febrile illness caused by the dengue virus, which now affects over 125 countries worldwide¹. According to the World Health Organization (WHO), confirmed dengue cases increased from 505,430 in 2000 to 5.2 million in 2019²⁻⁵. It is estimated that 300 million people contract dengue annually, with 100 million of these cases reported clinically. Additionally, 4 billion people are at risk of infection¹.

The belief that dengue is limited to tropical and subtropical regions is evolving, as recent reports show a global rise in cases^{3,5,6}. The dengue virus is transmitted from one person to another through bites from *Aedes* spp. mosquitoes. In the past decade, however, these mosquitoes have expanded into new regions, including areas that were previously free from dengue. Factors such as rapid transportation, increased travel, and the mosquitoes' ability to adapt to new environments have played a significant role in the spread of the disease⁷.

Bangladesh is considered an endemic region for dengue fever^{4,6}, with the first case reported in early 2000³⁻⁶. Since then, outbreaks have occurred regularly in the country. However, insufficient and ineffective surveillance systems have likely resulted in an underreporting of the actual number of cases and fatalities. Significant outbreaks, such as those in 2019 with 101,500 cases and in 2023 with 321,073 cases, have affected most of the previously non-endemic areas, presenting a major public health threat^{3,6}. Following COVID-19, dengue has emerged as a critical health issue, causing 1,705 deaths in 2023 alone. Many areas that were once free of dengue are now experiencing high transmission rates. Without effective treatment and prevention strategies, the continued spread of dengue poses a serious global health concern⁸.

The dengue virus is a positive-sense, single-stranded RNA virus with a genome approximately 11,000 bases long. Its genome encodes three structural proteins—capsid protein (C), membrane protein (M), and envelope protein (E)—as well as seven non-structural proteins: NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5. The dengue virus is classified into four distinct serotypes: DENV-1, DENV-2, DENV-3, and DENV-4. Each serotype is further subdivided into various genotypes based on genetic and phylogenetic analysis. However, there is a significant lack of genotypic surveillance data for the dengue virus in Bangladesh^{7,9}.

According to the WHO dengue case classification², dengue cases are categorized into three types: dengue without warning signs, dengue with warning signs, and severe dengue. Symptomatic infections can present as undifferentiated fever (viral syndrome), dengue fever syndrome (DFS), or dengue hemorrhagic fever (DHF).

In many DFS cases, unusual bleeding may occur, although some cases of DFS do not involve hemorrhaging. DHF can be further divided into cases with or without shock, with dengue shock syndrome (DSS) representing the more severe form¹⁰.

Most cases are mild, with infections that are either asymptomatic or cause mild symptoms. Common symptoms include fever (104°F), headache, muscle pain, pain behind the eyes, joint pain, nausea, vomiting, rash, and swollen glands. In severe cases, symptoms may include intense abdominal pain, persistent vomiting, difficulty breathing, bleeding from the gums or nose, blood in vomit or stool, restlessness, excessive thirst, pale and cold skin, and weakness^{5,10}.

Before 2002, dengue type 3 (DEN3) was commonly reported, and its re-emergence was observed again in 2017. This was followed by a sharp increase in dengue cases at the start of the monsoon season in 2018. As the outbreak expanded, a higher number of dengue cases and deaths were reported compared to the past 15 years. From 2013 to 2016, DEN1 and DEN2 were the most predominant strains, whereas DEN3 and DEN4 have become more common in recent years^{6,11}. Notably, co-detection of multiple dengue serotypes, such as DEN2 and DEN3, DEN1 and DEN3, or DEN1, DEN2, and DEN3, was found in a significant proportion of cases. The cross-reactivity of antibodies may increase the risk of severe dengue infection when DEN3 and DEN4 are co-detected¹². So this study aimed to investigate the demographic, clinical, and laboratory characteristics of dengue patients' of 2024 outbreak, focusing on gender differences and the role of platelet count in disease severity¹³.

Methodology

Study population and sample collection: This was a prospective study carried out in the department of virology in NILMRC, Dhaka, Bangladesh in the period of July to November 2024. Study population was all the confirmed dengue patient admitted in medicine ward of AMZ Hospital within this period fulfilling the inclusion and exclusion criteria and also outdoor patients of NILMRC. With all aseptic precaution blood samples (5 ml) were collected from those patients for further investigations. For data collection, a structured questionnaire was used, and after meeting the inclusion and exclusion criteria, informed written consent was obtained from the parents. A total of 146 samples were included in this study. All confirmed dengue cases, regardless of sex or ethnicity, were enrolled. Detailed histories of dengue infections and thorough clinical examinations were conducted. Dengue cases were defined and classified according to the National Guideline

for Clinical Management of Dengue Syndrome, 4th Edition (2018, revised). Patients were categorized into three groups: dengue fever (DF, including classical dengue and dengue with unusual hemorrhaging), dengue hemorrhagic fever (DHF, with or without shock), and expanded dengue syndrome (EDS). Adult people with NS1 antigen Positive were included in this study. Patients suffering from autoimmune disease or taking Immunosuppressive drugs were excluded from the study. The cost of the study was (240, 58.8%) was (>15,000) Bangladeshi taka (Table 1).

Serological tests: Laboratory investigations including a complete blood count (using the Sysmex XN 2000™ Hematology Autoanalyzer, Sysmex Corporation), serum alanine aminotransferase (ALT), aspartate amino transferase (AST), S creatinine, S albumin were performed on patient admission, and a complete blood count was repeated every alternate day to track hematocrit and platelet count changes. The definition of thrombocytopenia was a platelet count of 100,000 platelets/ μ L or less; leukopenia was a white blood cell count 5000cells/ μ L, a raised hematocrit was >48%, or $\geq$ 20% increase than the baseline, a raised ALT/AST level was >50IU/L¹³. After getting proper history and clinical findings appropriate management was given according to the protocol for Dengue management for children. Immediate clinical examination was performed in every patient with special attention to fever, vital signs, urine output, rash, organomegaly and, when needed, organ function. All patients were followed up daily until discharge.

RNA Extraction: Total RNA was extracted from samples obtained from patients who were dengue NS1 positive the Viral RNA Mini Kit (Qiagen, Crawley, UK), and samples were then stored at -80°C until real-time RT-PCR for serotyping was carried out.

Dengue serotyping by Multiplex Real-time Reverse Transcriptase-Polymerase Reaction: Altona RealStar® Dengue Type RT-PCR Kit 1.0 (Hamburg Germany) was used for the detection of DENV serotype. In multiplex reaction mixtures, Master A contain specific primer for DENV1 and DENV4 and Master B contain specific primer for DENV2 and DENV3. Reverse transcription of 20 min at 55°C was followed by 45 cycles of amplification in a Quant studio 5 real-time Detection System. In case of PCR result interpretation, Cycle Threshold (Ct) 38 was evaluated as positive and Ct value (38 was negative).

Statistical analysis: Statistical analysis was performed by Windows based software named as Statistical Package for Social Science (SPSS), versions 22.0 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.).

Ethical approval: The Institutional Review Board (IRB) of the NILMRC granted ethical permission (2024:0206). All participants provided their written, fully informed consent,

together with the parents of any minors. The doctors and the medical technologist collected personal information and other relevant medical data using a semi-structured questionnaire.

Results

Out of 146 total sample, 84 patients (57.9%) were male, while 62 patients (42.1%) were female. This indicates a slightly higher prevalence of dengue among male patients compared to female patients. presents the age group distribution of dengue patients by gender. The largest proportion of patients falls within the 16-25 age group (30.1%), followed closely by the 26-35 age group (29.5%). The youngest group (0-15 years) accounted for 17.1% of the total sample, while the older age groups had smaller representations: 11.0% in the 36-45 age group, 4.8% in the 46-55 age group, and 7.5% in the >56 age group. A higher number of male patients (56.2%) was observed compared to female patients (43.8%), with the male patients being predominant across all age groups (Figure I).

Analyzing the clinical features of 146 dengue patients, categorized by age group and gender, with corresponding p-values. Fever was reported by all 146 patients, with no significant gender differences (p-value not provided). Cough was reported by 55 patients (37.7%), with males (30) more frequently experiencing it than females (25), and a significant gender difference ($p = 0.05$). Headache was reported by 115 patients (78.8%), with males (91) more affected than females (24), and a significant gender difference ($p = 0.02$). Myalgia was reported by 108 patients (74.0%), with more males (78) than females (30), showing a significant gender difference ($p = 0.03$). Bleeding history was reported by only 9 patients (6.2%), with no significant gender or age differences ($p = 0.10$). Rash was observed in 72 patients (49.3%), with more females (38) experiencing it than males (34), showing a significant gender difference ($p = 0.01$). Nausea/vomiting was reported by 61 patients (41.8%), with more females (28) than males (33), with a marginal gender difference ($p = 0.05$). Fatigue/weakness was reported by 92 patients (63.0%), with more males (52) than females (40), showing a significant gender difference ($p = 0.03$). Joint pain was reported by 108 patients (74.0%), with more males (82) than females (26), with a significant gender difference ($p = 0.04$). Abdominal pain was reported by 49 patients (33.6%), with more males (30) than females (19), showing a marginal gender difference ($p = 0.07$). Low platelet count was reported by 38 patients (26.0%), with more males (25) than females (13), and a significant gender difference ($p = 0.02$). Liver enlargement was reported by 19 patients (13.0%), with no clear gender differences

($p = 0.06$). Complications like DHF/DSS were experienced by 12 patients (8.2%), with more males (8) than females (4), showing a significant gender difference ($p = 0.03$). Overall,

the table highlights varying clinical patterns based on age and gender in dengue patients, with significant gender differences in several features (Table 1).

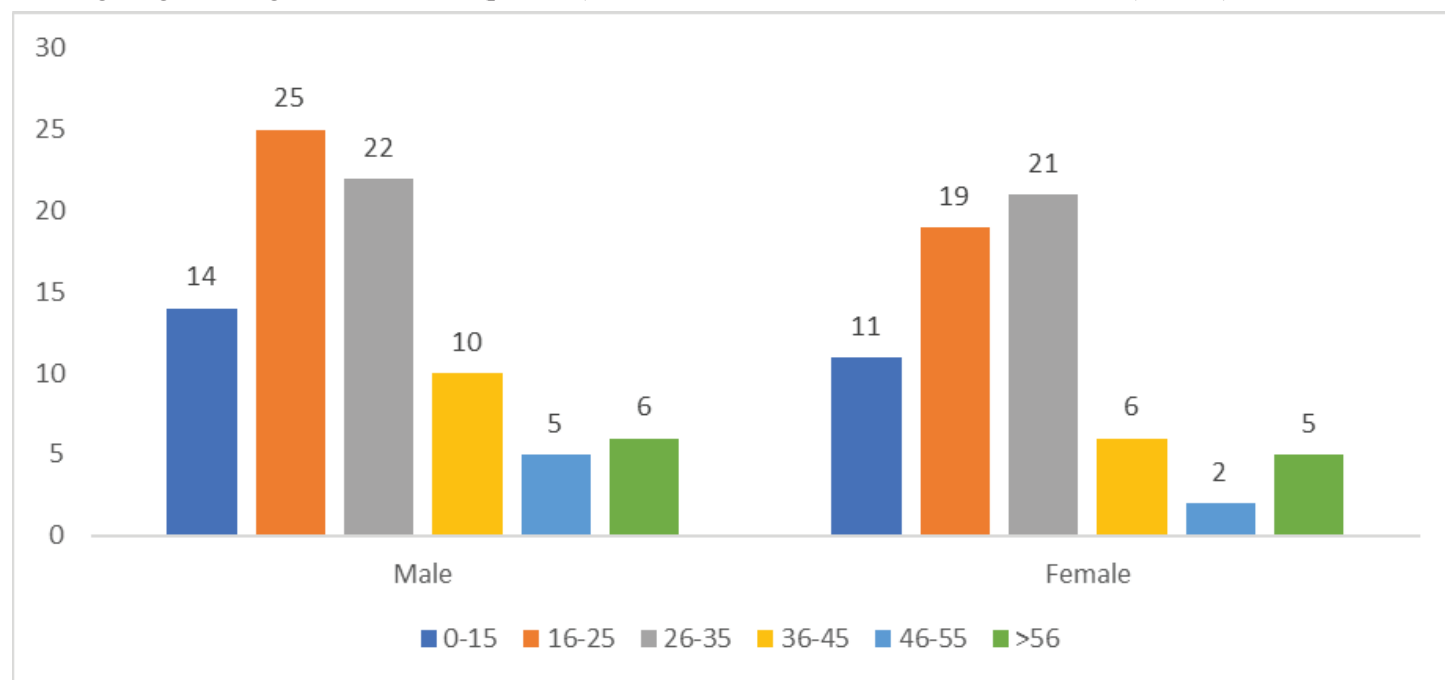


Figure I: Age Group Distribution of Dengue Patients by Gender

Table 1: Clinical Features of Dengue Patients (n=146) by Age Group and Gender

Clinical Feature	0-15	16-25	26-35	36-45	46-55	>56	Male	Female	P value
Fever	25	44	43	16	7	11	82	64	-
Cough	6	8	11	15	4	11	30	25	0.05
Headache	12	22	25	28	11	9	91	24	0.02
Myalgia	13	21	25	27	12	5	78	30	0.03
Bleeding History	1	1	2	3	1	1	4	5	0.10
Hospitalized History (Indoor)	1	3	2	1	5	3	12	7	0.04
Rash	12	7	10	7	8	4	34	38	0.01
Nausea/Vomiting	5	10	12	13	11	6	33	28	0.05
Fatigue/Weakness	19	23	28	21	14	6	52	40	0.03
Joint Pain	13	19	23	27	13	9	82	26	0.04
Abdominal Pain	2	4	4	6	9	3	30	19	0.07
Low Platelet Count	10	5	3	3	5	2	25	13	0.02
Liver Enlargement	1	3	2	1	5	3	12	7	0.06
Complications (DHF/DSS)	1	1	2	3	1	2	8	4	0.03

Chi-square test was performed to see the level of significance

All patients tested positive for NS1, indicating the sensitivity of this diagnostic marker. The majority of patients (84.2%) had platelet counts greater than 100,000 cells/ μ L, while

15.8% had lower platelet counts, reflecting the thrombocytopenia often associated with dengue (Table 2).

Table 2: Investigation Findings of Dengue Patients (n=146)

Variable	Frequency	Percent	P value
NS1			
• Positive	146	100.0	-
• Negative	0	0.0	-
Platelet Count (cells/μL)			
• >100,000	123	84.2	0.04
• $\leq$ 100,000	23	15.8	-
HCT (%)			
• >48%	7	4.8	0.02
• $\leq$ 48%	139	95.2	-
WBC (cells $\times 10^3$ cells/μL)			
• >5	92	63.0	0.05
• $\leq$ 5	54	37.0	-
PCR Serotype			
• DENV-1	0	0.0	0.001
• DENV-2	89	61.0	-
• DENV-3	23	15.8	-
• DENV-4	6	4.1	-
• DENV-1, DENV-3	9	6.2	-
• DENV-2, DENV-3	18	12.3	-
• DENV-2, DENV-4	1	0.7	-
Dengue IgG			
• Positive	105	71.9	0.04
• Negative	41	28.1	-
Liver Enzyme (AST, ALT)			
• AST > 100 IU/L	62	42.5	0.01
• ALT > 100 IU/L	54	37.0	0.03
• Normal	30	20.5	-
Serum Creatinine (mg/dL)			
• >1.2	18	12.3	0.04
• $\leq$ 1.2	128	87.7	-
Dengue Hemorrhagic Fever (DHF)			
• Yes (Criteria for DHF)	14	9.6	0.02
• No (No DHF)	132	90.4	-
Thrombocytopenia Severity			
• Severe (<50,000)	5	3.4	0.01
• Moderate (50,000–100,000)	18	12.3	-
• Mild (>100,000)	123	84.2	-
Albumin Levels (g/dL)			
• <3.0	25	17.1	0.03
• $\geq$ 3.0	121	82.9	-
Dengue Shock Syndrome (DSS)			
• Yes (Shock Present)	8	5.5	0.04
• No (No Shock)	138	94.5	-

Chi-square test was performed to see the level of significance

A significant majority (95.2%) had hematocrit levels $\leq$ 48%, with only 4.8% exceeding this threshold, suggesting few severe cases. WBC counts were elevated ($>5,000$ cells/ μ L) in 63% of patients. The predominant serotype was DENV-2 (61%), followed by DENV-3 (15.8%) and DENV-4 (4.1%). Liver enzymes, particularly AST and ALT, were elevated in a significant proportion of patients, with 42.5% showing AST levels >100 IU/L and 37% with ALT levels >100 IU/L. Additionally, serum creatinine levels >1.2 mg/dL were found in 12.3% of cases. Complications such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) were identified in 9.6% and 5.5% of patients, respectively. The majority (84.2%) of patients had mild thrombocytopenia, and 17.1% had albumin levels <3.0 g/dL. Statistically significant p-values were observed for several key parameters, including platelet count ($p=0.04$), liver enzymes (AST: $p=0.01$, ALT: $p=0.03$), and thrombocytopenia severity ($p=0.01$) (Figure II A, IIB, IIC).

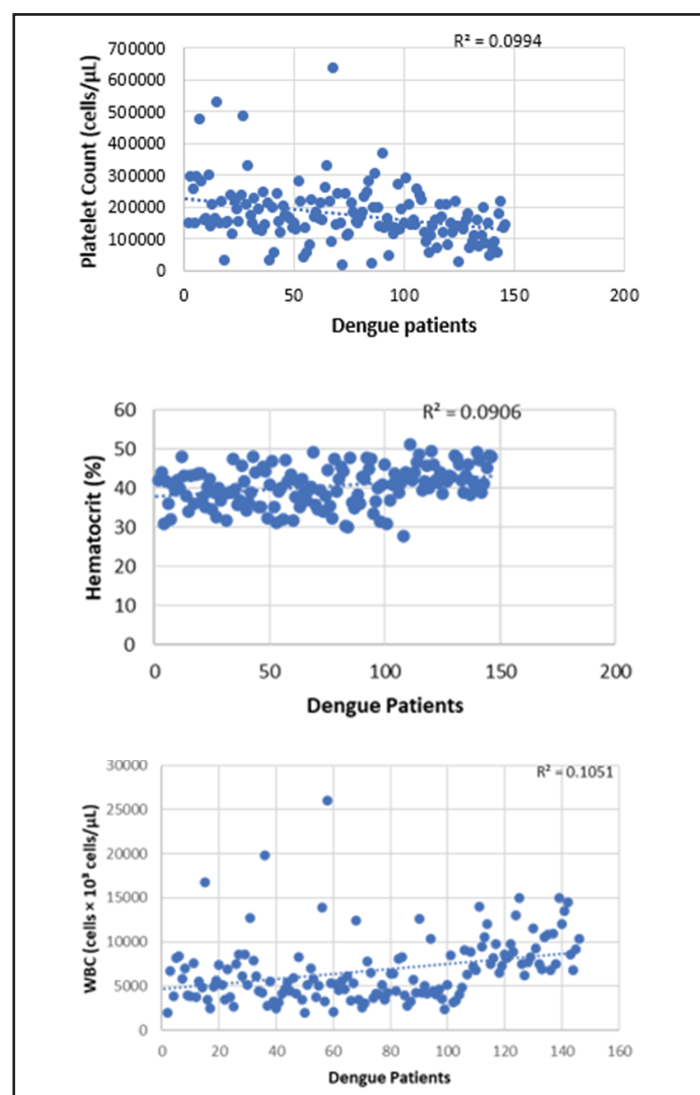
**Figure II (A): Platelet Distribution (B): Hematocrit Distribution and (C): WBC Distribution of Dengue Patients (n=146)**

Table 3 presents the correlation between platelet count and severity markers in dengue patients. The data reveals that patients with a platelet count of $\leq 100,000$ cells/ μ L have a significantly higher incidence of severe complications compared to those with platelet counts $>100,000$ cells/ μ L. Specifically, 60.9% of patients with a platelet count $\leq 100,000$ cells/ μ L had elevated AST levels (>100 IU/L), compared to 35.8% of those with platelet counts $>100,000$ cells/ μ L ($p<0.05$). Similarly, 55.2% of patients with a platelet count $\leq 100,000$ cells/ μ L exhibited elevated ALT levels (>100 IU/L), while only 30.1% of patients with

platelet counts $>100,000$ cells/ μ L had elevated ALT levels ($p<0.05$). The incidence of hematocrit rise more than 20.0%, a key indicator of severe dengue, was also higher in the low platelet count group (15.4% vs. 7.4%, $p<0.05$), as well as the occurrence of complications such as Dengue Hemorrhagic Fever (DHF) or Dengue Shock Syndrome (DSS), with 25.0% of patients in the low platelet count group experiencing complications, compared to only 7.3% in the high platelet count group ($p<0.05$). These findings underscore the association between lower platelet counts and more severe clinical outcomes in dengue patients (Table 3)

Table 3: Correlation of Platelet Count with Severity Markers Lorem ipsum

Platelet Count	Liver Enzyme (AST > 100 IU/L) (%)	Liver Enzyme (ALT > 100 IU/L) (%)	Hematocrit Rise >20% (%)	Complications (DHF/DSS) (%)	Dengue Hemorrhagic Fever (DHF)
>100,000 cells/ μ L	35.8	30.1	7.4	7.3	8.1
$\leq 100,000$ cells/ μ L	60.9	55.2	15.4	25.0	16.7
Total	42.5	37.0	8.9	9.6	9.6

Discussion

Dengue fever, a mosquito-borne viral illness, continues to be a significant public health concern in many tropical and subtropical regions worldwide. The present study aimed to evaluate the clinical and demographic characteristics of dengue patients, with a focus on gender differences, age distribution, clinical features, and laboratory findings. The findings reveal several important insights into the patterns and severity of dengue infection, as well as the influence of gender, age, and platelet count on clinical outcomes. In this study, the male population (57.9%) slightly outnumbered the female population (42.1%), suggesting a marginally higher prevalence of dengue among male patients. This aligns with previous studies, which have indicated a greater susceptibility or more frequent diagnosis of dengue in males compared to females¹⁴. Additionally, the age distribution of the patients showed that the largest proportion of dengue cases occurred in the 16-25 (30.1%) and 26-35 (29.5%) age groups, consistent with reports indicating that adults in their late teens to early thirties are often at higher risk of contracting the disease¹⁵. A smaller proportion of patients were observed in older age groups, with those over 45 years of age representing less than 25% of the total sample, similar to findings in other studies¹⁶. A detailed analysis of clinical features revealed several gender-based differences in symptom presentation. Fever

was reported universally, with no significant gender differences, but several other symptoms, including cough, headache, myalgia, fatigue/weakness, and joint pain, showed significant gender differences. Males were more likely to report symptoms like cough ($p = 0.05$), headache ($p = 0.02$), myalgia ($p = 0.03$), fatigue/weakness ($p = 0.03$), and joint pain ($p = 0.04$), while females more frequently reported rash ($p = 0.01$) and nausea/vomiting ($p = 0.05$). The higher incidence of joint pain and myalgia in males is consistent with studies suggesting a higher severity of musculoskeletal symptoms in male patients¹⁷. Similarly, the observation that females reported more rash and nausea/vomiting might be attributed to differences in immune response between genders, as female patients often exhibit a more robust immune response to infections, which could result in increased systemic symptoms like rash¹⁶. The association of gender with clinical outcomes such as bleeding history and complications like Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) is noteworthy. Males had a higher prevalence of severe complications, with 8.2% of the patients experiencing DHF/DSS, and a significant gender difference ($p = 0.03$). This finding is consistent with reports that suggest male patients may experience more severe outcomes, including bleeding and shock, compared to females¹⁸.

The laboratory findings from this study confirm the typical diagnostic markers associated with dengue infection. All patients tested positive for the NS1 antigen, underscoring its sensitivity as a diagnostic marker for early detection of dengue¹⁹. A significant majority of patients (84.2%) had platelet counts above 100,000 cells/ μ L, which is consistent with previous studies showing thrombocytopenia as a hallmark of dengue infection²⁰. Additionally, elevated liver enzymes (AST and ALT) were observed in a substantial proportion of patients, with 42.5% showing elevated AST levels >100 IU/L, and 37% with ALT levels >100 IU/L. These findings are consistent with prior studies suggesting hepatic involvement in dengue infection, as elevated liver enzymes are often observed in patients with dengue fever²¹. An important observation from the laboratory data is the significant association between lower platelet counts ($\leq$ 100,000 cells/ μ L) and severe clinical outcomes. Patients with lower platelet counts were more likely to experience elevated AST and ALT levels, hematocrit rises, and complications such as DHF and DSS. This aligns with previous research, which has established thrombocytopenia as a key predictor of severe dengue disease and its complications²². The correlation between platelet counts and clinical severity emphasizes the need for careful monitoring of platelet levels in dengue patients, as lower platelet counts are indicative of a higher risk for developing severe manifestations such as bleeding, shock, and multi-organ involvement.

Conclusion

This study provides valuable insights into the demographic and clinical characteristics of dengue patients, highlighting important gender and platelet count differences in the presentation and severity of the disease. Males appear to be more frequently affected by certain clinical symptoms, including musculoskeletal pain and fatigue, and are also more likely to develop severe complications such as DHF and DSS. The findings also underscore the importance of platelet count as a key marker for predicting severe dengue outcomes. These results can guide healthcare providers in managing dengue patients more effectively, particularly in regions with endemic dengue transmission.

Future studies should focus on the mechanisms behind the observed gender differences in dengue infection and explore the role of immune responses in the disease's pathogenesis. Additionally, further investigation into the relationship between laboratory markers, such as platelet count and liver enzyme levels, and clinical outcomes could help refine prognostic tools for dengue.

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Author Contributions: Conceptualization & Supervision: AA; Methodology: TN, TIR; Data curation and Formal analysis: NH; Writing first draft: Arifa Akram; Writing- editing and revision: Md. A R

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