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EVALUATION OF LIVER ENZYME ABNORMALITIES IN DENGUE FEVER: A COMPARATIVE CLINICAL STUDY OF 2024 AND 2025 IN ISLAMABAD

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Abstract

Background: *Dengue fever represents an escalating public health burden in Pakistan, with national case counts exceeding 20,000 in 2024 and Islamabad consistently reporting substantial annual epidemic activity. Hepatic dysfunction, marked by elevation of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), is among the most clinically significant systemic complications of dengue infection and can progress to acute liver failure in severe presentations.*

Objective: *This study compared the prevalence, severity, and pattern of hepatic dysfunction between two consecutive dengue epidemic seasons in Islamabad, Pakistan, and determined whether hepatic involvement changed significantly between 2024 and 2025.*

Methods: *A retrospective comparative cohort design was used, incorporating 224 NS1-positive dengue patients (112 per year) from Islamabad. Age, gender, NS1 antigen value, ALT, and AST were recorded for each patient. Data were analyzed in SPSS using descriptive statistics, the Shapiro-Wilk test, the Independent Samples t-test, the Mann-Whitney U test, the chi-square test, Fisher's exact test, and Pearson correlation, with $p < .05$ considered statistically significant. Ethical approval was granted by the Health Sciences/Technology Ethics Review Committee of National Skills University, Islamabad, and a waiver of informed consent was obtained for this retrospective, de-identified dataset.*

Results: *Mean ALT rose from 43.71 ± 22.28 U/L in 2024 to 55.55 ± 44.66 U/L in 2025 ($t(222) = -2.512$, $p = .013$, 95% CI for the mean difference [2.55, 21.13], $d = 0.34$), and mean AST rose from 48.99 ± 35.17 U/L to 72.31 ± 77.01 U/L ($t(222) = -2.915$, $p = .004$, 95% CI [7.55, 39.09], $d = 0.39$). Moderate ALT elevation increased more than fourfold (4.5% to 20.5%; $\chi^2(2) = 13.333$, $p = .001$, Cramér's $V = 0.24$), and moderate AST elevation doubled (12.5% to 25.0%; $\chi^2(3) = 11.595$, $p = .009$, Cramér's $V = 0.23$); one case of severe AST elevation (>310 U/L) occurred in 2025 versus none in 2024. Mann-Whitney U testing showed no significant difference in ALT ($p = .916$) or AST ($p = .665$) rank distributions, and median values were essentially unchanged between years, indicating that the mean-level worsening was driven by a growing subgroup of more severely affected patients rather than a shift across the whole cohort. NS1 antigen level correlated weakly with ALT ($r = .186$, $p = .049$) and AST ($r = .190$, $p = .045$) in the 2024 cohort only; this association was absent in 2025. ALT and AST were strongly correlated with one another in both years ($r = .955–.970$, $p <$*

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.001). Gender distribution did not differ significantly between years ($\chi^2(1) = 1.956$, $p = .162$), although the 2025 cohort included a wider age range with more pediatric and elderly patients.

Conclusion: *Hepatic dysfunction among dengue patients in Islamabad worsened significantly between 2024 and 2025, with a growing minority of patients developing moderate-to-severe transaminase elevation. These findings support incorporating routine liver function testing into standard dengue case management in Islamabad, and potentially more broadly across Pakistan, to enable earlier identification of patients at risk of hepatic complications.*

1. Introduction

Dengue fever, caused by four antigenically distinct serotypes of the dengue virus (DENV-1 to DENV-4) and transmitted primarily by the Aedes aegypti mosquito, is one of the most rapidly spreading vector-borne infectious diseases in the world. Its expansion beyond traditional endemic boundaries has been driven by climate change, unplanned urbanization, increased global travel, and the introduction of the virus into immunologically naive populations. The World Health Organization estimates that approximately 390 million dengue infections occur globally each year, of which around 96 million manifest clinically, with the Asia-Pacific region bearing the greatest burden (World Health Organization [WHO], 2024). Modeling work by Bhatt et al. (2013) placed roughly half of the world's population at risk of dengue transmission, and the geographic spread of the disease has been linked to the international travel and trade networks that move both the virus and its mosquito vector into previously unaffected regions (Wilder-Smith & Gubler, 2008). The year 2024 recorded the highest global dengue caseload on record, with more than 14.4 million cases reported to the WHO.

In Pakistan, dengue has evolved over the past two decades from a sporadic urban illness into a recurring nationwide epidemic. Since the first laboratory-confirmed case in Karachi in 1994, the disease has spread across all provinces, with large-scale outbreaks recorded annually since 2005, typically peaking between August and November; Humayoun et al. (2010) documented multiple co-circulating serotypes and a high frequency of dengue hemorrhagic fever during the 2008 Lahore outbreak, an early indicator of the disease's evolving severity profile in the country. More recently, Zohra et al. (2024) characterized the demographic and clinical features of dengue across a large national sample of 25,682 patients, reporting a male predominance consistent with patterns observed in the present study. Surveillance data from the National Institute of Health (NIH) Pakistan indicate that confirmed dengue cases rose to more than 20,000 in 2024, roughly sixfold higher than in 2020. Genomic surveillance conducted by NIH Pakistan in 2024 identified the DENV-2 Cosmopolitan genotype (clade IV-A) as the dominant circulating strain, accounting for 93% of confirmed cases; this serotype has been associated in the literature with more pronounced hepatic involvement than other dengue serotypes (National Institute of Health [NIH] Pakistan, 2025).

Islamabad, the federal capital and the setting of the present study, is a persistent dengue hotspot. Its dense urban development, ongoing construction activity, standing water sources, and proximity to the Margalla Hills create favorable conditions for Aedes aegypti breeding and disease transmission. Because both cohorts analyzed in this study were drawn from Islamabad, the findings carry direct relevance for local clinical practice and health system planning.

Hepatic dysfunction is among the most clinically consequential systemic complications of dengue infection. The liver is a principal target organ in dengue: the virus directly infects hepatocytes and Kupffer cells, producing cytopathic hepatocellular injury, while a concurrent surge of pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), and interleukin-6 (IL-6) contributes an immune-mediated pathway of hepatocellular damage (Leowattana & Leowattana, 2021). This combined

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injury releases alanine aminotransferase (ALT) and aspartate aminotransferase (AST) into the circulation, producing a clinical spectrum that ranges from asymptomatic transaminase elevation, the most common presentation, to fulminant hepatic failure with encephalopathy and coagulopathy in the most severe cases. In secondary dengue infections, pre-existing non-neutralizing antibodies can further enhance viral replication and amplify the cytokine response through antibody-dependent enhancement (ADE), a mechanism associated with more severe clinical outcomes, including greater hepatic involvement (Halstead, 2007).

Several recent studies provide direct points of comparison for the present analysis. Among Pakistani cohorts, Khan et al. (2025), studying 135 dengue patients at Lady Reading Hospital, Peshawar, reported hepatic dysfunction in 53.3% of patients, with 8.9% classified as severe and an associated 3.7% mortality confined to the severe subgroup. Jamil et al. (2025), in a Multan cohort, found that a combined AST plus ALT value exceeding 300 U/L was significantly associated with mortality. Sadiq et al. (2024), studying 60 pediatric dengue patients at a Federal Government Polyclinic in Islamabad, reported AST elevation in 96.7% of patients by day 7 of illness, highlighting the pediatric population as particularly susceptible to hepatic involvement. Beyond Pakistan, a Kolkata-based study using an ELISA-based diagnostic platform reported a positive correlation between NS1 antigen level and transaminases (r = .34–.41) (Palmal et al., 2023), while Senaratne et al. (2016) linked elevated interleukin-2, an immune-activation marker, to more severe hepatic derangement in dengue patients from Sri Lanka. At the international level, the systematic review by Campana et al. (2024) estimated hepatic involvement in 14–80% of dengue patients across included studies and noted that AST elevation consistently exceeded ALT elevation, particularly in more severe disease, while the meta-analysis by Kalluru et al. (2023) reported mean ALT values of 60–120 U/L in moderate dengue, rising above 200 U/L in severe disease, situating hepatic enzyme elevation as a graded marker of disease severity across diverse populations.

Taken together, the existing literature establishes that hepatic dysfunction is a common and clinically important complication of dengue infection, with international prevalence estimates ranging widely (14–80%) and Pakistani single-season studies reporting hepatic dysfunction in roughly half to two-thirds of patients. However, most Pakistani studies on this topic remain single-season, single-center, cross-sectional analyses, and comparative, year-on-year evidence from Islamabad specifically is limited. This gap has practical consequences. First, the severity of dengue-associated liver injury is not static: it is influenced by the dominant circulating serotype, the proportion of secondary infections (and the associated risk of antibody-dependent enhancement), host immune status, and patient demographics, all of which can shift the clinical picture from one epidemic season to the next (Halstead, 2007). Second, Pakistan currently lacks standardized national guidance mandating routine liver function testing in dengue case management; comparative evidence of a worsening hepatic trend would strengthen the scientific rationale for such guidance. Third, Islamabad remains under-represented in the national literature on dengue-related hepatic dysfunction relative to Lahore, Karachi, and Peshawar, limiting the direct applicability of existing evidence to the capital.

This study therefore compared two annual cohorts of 112 NS1-positive dengue patients each, from the 2024 and 2025 epidemic seasons in Islamabad, to determine whether the incidence and severity of hepatic dysfunction, indexed by ALT and AST, changed between the two years. The primary research question, as approved by the Health Sciences/Technology Ethics Review Committee (HST-ERC) of National Skills University, Islamabad, was: has the incidence, severity, or pattern of hepatic dysfunction in laboratory-confirmed dengue patients changed significantly between 2024 and 2025 in Islamabad, Pakistan? The primary objective was to establish whether the incidence and degree of ALT- and AST-defined hepatic dysfunction changed between the two

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years; secondary objectives were to compare the incidence of hepatic dysfunction (ALT >41 U/L and/or AST >31 U/L) between cohorts, to compare severity-category distributions (normal, mild, moderate, severe), and to compare mean ALT and AST using both the Independent Samples t-test and the Mann-Whitney U test. The null hypothesis (H_0) stated that no statistically significant difference existed in mean ALT, mean AST, or the distribution of hepatic dysfunction severity between 2024 and 2025 ($p > .05$); the alternative hypothesis (H_1) proposed that these measures differed significantly between the two years ($p \leq .05$).

2. Materials and Methods

2.1 Study Design

This study used a retrospective comparative cohort design, in which previously recorded laboratory data from dengue patients diagnosed in 2024 and 2025 were extracted and compared. No new patient contact, sample collection, or clinical intervention occurred. The design permitted a direct comparison of dengue-related hepatic dysfunction across two consecutive epidemic seasons using standardized biochemical markers. The study protocol was approved by the Health Sciences/Technology Ethics Review Committee (HST-ERC) of National Skills University, Islamabad, prior to data collection, and all data-handling procedures (anonymization, password-protected storage, and non-disclosure of the source hospital) followed the approved protocol throughout.

2.2 Study Setting and Duration

The study was conducted in Islamabad, Pakistan. The 2024 dataset was compiled directly by the research team from laboratory records of dengue NS1-positive patients during the 2024 outbreak. The 2025 dataset was obtained from a tertiary care hospital in Islamabad under a formal data-sharing and confidentiality agreement; per the approved protocol and the agreement's terms, the identity of the collaborating hospital is not disclosed. Data collection, validation, and statistical analysis were conducted between early and mid-2026, following ethics approval.

2.3 Study Population, Sample Size, and Sampling

The study population comprised male and female patients of all ages diagnosed with dengue fever in Islamabad in 2024 or 2025, confirmed by an NS1 antigen. A consecutive sampling approach was used: all eligible patients with complete records for the five study variables (serial number, age, gender, NS1, ALT, AST) were included until the target sample size per year was reached.

Sample size was determined using standard biostatistical parameters for a two-group independent-means comparison, the primary planned analysis being the Independent Samples t-test comparing ALT and AST between cohorts: a two-tailed significance level of .05 ($Z = 1.96$), statistical power of 80% ($Z = 0.84$), an anticipated between-group mean difference of approximately 15 U/L (based on published Pakistani dengue studies reporting mean ALT of 85–107 U/L), and an assumed standard deviation of approximately 35 U/L, informed by prior Pakistani clinical studies. This standard deviation assumption was checked against a pilot subset of 20 patient records (10 per year) these 20 pilot records were a subset of, and are included in, the final analytic sample, which showed an observed ALT standard deviation of approximately 33–37 U/L, consistent with the assumed value. Applying the formula $n = 2 \times [(Z_a/_2 + Z^b)^2 \times \sigma^2] \div \delta^2$ yielded a minimum required sample of approximately 86–110 patients per group. A final sample of 112 patients per year (224 total) was selected to provide a margin above this minimum and to accommodate any records excluded during quality control.

2.4 Inclusion and Exclusion Criteria

Patients were included if they had a laboratory-confirmed NS1 antigen value greater than 1.1 OD ratio units, were of any age or gender, had complete data for all five study variables, and were treated in Islamabad during the 2024 or 2025 dengue seasons. Patients were excluded if their NS1 value was 1.0 or below (dengue-negative per study criteria), if any of the NS1, ALT, or AST values were missing, if they had known pre-existing chronic liver disease (e.g., hepatitis B, hepatitis C,

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or cirrhosis), if they were taking hepatotoxic medication at the time of dengue diagnosis, or if their record was a duplicate entry.

2.5 Study Variables and Severity Classification

The independent variable was study year (2024 vs. 2025). The principal dependent variables were serum ALT and AST concentration (U/L); secondary dependent variables were a derived categorical severity classification and NS1 antigen level (OD ratio, ELISA-based), used as an indicator of viral load. Hepatic dysfunction was defined as ALT greater than 41 U/L and/or AST greater than 31 U/L. Severity was classified using multiples of the upper limit of normal (ULN): normal (ALT ≤41 U/L; AST ≤31 U/L), mild (1–3× ULN; ALT 42–123 U/L, AST 32–93 U/L), moderate (3–10× ULN; ALT 124–410 U/L, AST 94–310 U/L), and severe (>10× ULN; ALT >410 U/L, AST >310 U/L).

2.6 Data Collection and Quality Control

The 2024 dataset was compiled by the research team on a structured proforma capturing serial number, age, gender, NS1 value, ALT, and AST, and tabulated in Microsoft Excel. The 2025 dataset was received from the collaborating hospital in anonymized form under the data-sharing agreement, with all personal identifiers removed prior to handover. Before statistical analysis, a systematic quality control review confirmed that both cohorts contained 112 complete records each, with no missing values, no gaps in serial numbering, no duplicate records, no NS1 values outside the plausible range (>300), and no extreme ALT or AST values (>500 U/L) attributable to data-entry error.

2.7 Statistical Analysis

All 224 records were entered into a single SPSS data file (IBM SPSS Statistics, trial version), with both cohorts stacked vertically and distinguished by a grouping variable (Year: 1 = 2024, 2 = 2025). Descriptive statistics (mean, standard deviation, median, interquartile range, minimum, maximum) were computed for age, NS1, ALT, and AST in each cohort. The Shapiro-Wilk test assessed normality of ALT and AST distributions. Because both variables were non-normally distributed in both years, the Mann-Whitney U test was used as the primary non-parametric comparison of ALT and AST between cohorts, with the Independent Samples t-test performed as a supplementary parametric analysis given the relatively large per-group sample size (n = 112). The chi-square test compared categorical distributions (gender; age group; ALT and AST severity categories) between years, with Fisher's exact test applied where an expected cell count fell below 5. Effect sizes were computed alongside significance tests: Cohen's d for the t-tests (using the pooled standard deviation), and Cramér's V for the chi-square tests. Pearson correlation examined the linear relationship between NS1 antigen level and ALT/AST, computed for the combined dataset and separately for each year. A p-value below .05 was considered statistically significant throughout, consistent with the HST-ERC–approved protocol.

2.8 Ethical Considerations

The study protocol received full review and approval from the HST-ERC of National Skills University, Islamabad. Because the study used retrospective, de-identified data with no new patient contact, a waiver of informed consent was approved by the HST-ERC. All records were assigned unique study identifiers, and electronic data were stored in a password-protected file accessible only to the research team and supervisor. The source hospital for the 2025 dataset is not named, in accordance with the data-sharing and confidentiality agreement. No artificial intelligence tools were used in data collection or statistical analysis; all analyses were performed exclusively in SPSS.

3. Results

3.1 Descriptive Statistics and Cohort Characteristics

Table 1 summarizes the descriptive statistics for age, NS1 antigen level, ALT, and AST in each cohort. Mean age was comparable between years (38.74 ± 11.69 years in 2024; 37.48 ± 17.01 years in 2025), although the 2025 cohort showed a wider age range (5–77 years vs. 14–76 years), with

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a higher proportion of pediatric patients (9.8% vs. 1.8%) and elderly patients (10.7% vs. 1.8%); adults aged 18–60 years remained the majority in both years (96.4% in 2024; 79.5% in 2025). Mean ALT increased from 43.71 ± 22.28 U/L in 2024 to 55.55 ± 44.66 U/L in 2025, an increase of 11.84 U/L (+27.1%); mean AST increased from 48.99 ± 35.17 U/L to 72.31 ± 77.01 U/L, an increase of 23.32 U/L (+47.6%). Median ALT was unchanged between years (37.0 U/L), while median AST was marginally lower in 2025 (27.0 U/L) than in 2024 (28.0 U/L); both cohorts displayed right-skewed distributions in which the mean exceeded the median, reflecting a subset of patients with markedly elevated values.

Table 1. Descriptive statistics for age, NS1 antigen level, ALT, and AST, 2024 vs. 2025 (N = 224).

Variable	Year	N	Mean	SD	Median	IQR	Min	Max
Age (years)	2024	112	38.74	11.69	38.00	29.00–48.00	14	76
Age (years)	2025	112	37.48	17.01	37.00	24.00–51.25	5	77
NS1 (OD ratio)	2024	112	52.18	21.35	54.85	35.97–63.25	10.5	102.0
NS1 (OD ratio)	2025	112	53.87	31.69	55.95	28.15–75.30	3.14	231.0
ALT (U/L)	2024	112	43.71	22.28	37.00	29.00–55.00	14	140
ALT (U/L)	2025	112	55.55	44.66	37.00	22.00–78.00	10	199
AST (U/L)	2024	112	48.99	35.17	28.00	24.00–71.25	12	199
AST (U/L)	2025	112	72.31	77.01	27.00	23.00–96.00	12	312

Gender Distribution

Gender distribution did not differ significantly between years (Table 2a): 78 patients (69.6%) were male in 2024 and 67 (59.8%) in 2025, versus 34 (30.4%) and 45 (40.2%) female, respectively ($\chi^2(1) = 1.956$, $p = .162$, Cramér's V = 0.09, indicating a negligible association). The 2025 cohort also included a wider age spread, with more pediatric and elderly patients (Table 2b).

Table 2a. Gender distribution by year (N = 112 per year). Cramér's V = 0.09.

Characteristic	2024, n (%)	2025, n (%)	$\chi^2(1)$	p
Male	78 (69.6%)	67 (59.8%)	1.956	.162
Female	34 (30.4%)	45 (40.2%)	—	—

Table 2b. Age-group distribution by year (N = 112 per year). Cramér's V = 0.26 (small-to-moderate association).

Characteristic	2024, n (%)	2025, n (%)	$\chi^2(2)^a$	p^a
Pediatric (<18 y)	2 (1.8%)	11 (9.8%)	15.206	<.001
Adult (18–60 y)	108 (96.4%)	89 (79.5%)	—	—
Elderly (>60 y)	2 (1.8%)	12 (10.7%)	—	—

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3.2 Normality and Comparative Testing of ALT and AST

The Shapiro-Wilk test indicated non-normal distributions for ALT and AST in both cohorts (all $p < .001$). Given this, the Mann-Whitney U test was treated as the primary comparison, with the Independent Samples t-test conducted as a supplementary parametric analysis in view of the adequate per-group sample size. Table 3 presents both sets of results, together with effect sizes and confidence intervals 95% CI for (2025 − 2024) mean difference.

Table 3. Comparative testing of ALT and AST, 2024 vs. 2025. *p < .05.

Variable	Test	Statistic	df	N	p	95% CI (mean diff.)	Effect size	Interpretation
ALT (U/L)	Independent t-test	t = −2.512	222	—	.013*	[2.55, 21.13]	d = 0.34	Significant
AST (U/L)	Independent t-test	t = −2.915	222	—	.004*	[7.55, 39.09]	d = 0.39	Significant
ALT (U/L)	Mann-Whitney U	U = 6220.5	—	224	.916	—	—	Not significant
AST (U/L)	Mann-Whitney U	U = 6062.0	—	224	.665	—	—	Not significant

3.3 Severity Distribution of Hepatic Dysfunction

The distribution of ALT-based severity categories differed significantly between years (Table 4). Moderate ALT elevation (124–410 U/L) rose from 5 patients (4.5%) in 2024 to 23 patients (20.5%) in 2025, an increase of more than fourfold ($\chi^2(2) = 13.333$, $p = .001$, Cramér's V = 0.24); the mild category declined from 42.0% to 33.0%, and no severe ALT elevation occurred in either year.

Table 4. ALT severity distribution, 2024 vs. 2025 . Pearson $\chi^2(2) = 13.333$, $p = .001$; Cramér's V = 0.24.

ALT category	Range	2024, n (%)	2025, n (%)	Total
Normal	≤41 U/L	60 (53.6%)	52 (46.4%)	112
Mild	42–123 U/L	47 (42.0%)	37 (33.0%)	84
Moderate	124–410 U/L	5 (4.5%)	23 (20.5%)	28
Severe	>410 U/L	0 (0.0%)	0 (0.0%)	0
Total	—	112	112	224

The AST-based severity distribution likewise differed significantly between years (Table 5). Moderate AST elevation (94–310 U/L) doubled from 14 patients (12.5%) in 2024 to 28 patients (25.0%) in 2025, and one patient (0.9%) in 2025 had severe AST elevation (>310 U/L), compared with none in 2024 ($\chi^2(3) = 11.595$, $p = .009$, Cramér's V = 0.23; Fisher's exact test for the severe cell, $p = .500$, reported given an expected count below 5). The normal category was stable across years (58.0% vs. 59.8%).

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Table 5. AST severity distribution, 2024 vs. 2025. Pearson $\chi^2(3) = 11.595$, $p = .009$; Cramér's V = 0.23.

AST category	Range	2024, n (%)	2025, n (%)	Total
Normal	≤31 U/L	65 (58.0%)	67 (59.8%)	132
Mild	32–93 U/L	33 (29.5%)	16 (14.3%)	49
Moderate	94–310 U/L	14 (12.5%)	28 (25.0%)	42
Severe	>310 U/L	0 (0.0%)	1 (0.9%)	1

3.4 NS1 Antigen and Its Relationship with Liver Enzymes

Table 6 presents the Pearson correlation analysis; the correlation coefficient r itself serves as the effect size for each association. In the combined dataset (N = 224), NS1 antigen level was not significantly correlated with ALT (r = .031, p = .645) or AST (r = .021, p = .757). When analyzed by year, a weak but statistically significant positive correlation was present in 2024 between NS1 and ALT (r = .186, p = .049) and between NS1 and AST (r = .190, p = .045); this correlation was absent in 2025 (NS1 vs. ALT: r = −.027, p = .774; NS1 vs. AST: r = −.038, p = .688). ALT and AST were very strongly correlated with each other in both years (r = .970 in 2024; r = .955 in 2025; both p < .001).

Table 6. Pearson correlation of NS1 antigen level with ALT and AST. *p < .05.

Correlation	Dataset	r	p	N	Interpretation
NS1 vs. ALT	Combined	0.031	0.645	224	Not significant
NS1 vs. AST	Combined	0.021	0.757	224	Not significant
ALT vs. AST	Combined	0.955	<0.001	224	Highly significant
NS1 vs. ALT	2024 only	0.186	0.049	112	Weakly significant
NS1 vs. ALT	2025 only	−0.027	0.774	112	Not significant
NS1 vs. AST	2024 only	0.190	0.045	112	Weakly significant
NS1 vs. AST	2025 only	−0.038	0.688	112	Not significant
ALT vs. AST	2024 only	0.970	<0.001	112	Highly significant
ALT vs. AST	2025 only	0.955	<0.001	112	Highly significant

3.5 ALT versus AST: Comparative Magnitude of Change

The proportional increase in mean AST (+47.6%) exceeded that in mean ALT (+27.1%) between 2024 and 2025, and the 2025 cohort showed an AST-predominant pattern (mean AST 72.31 U/L vs. mean ALT 55.55 U/L). The overall hepatic dysfunction rate (ALT and/or AST above normal) ALT was 46.5% in 2024 and 53.5% in 2025 and AST was 42.0% in 2024 and 40.2% in 2025.

4. Discussion

4.1 Principal Findings

This comparative cohort study found that hepatic dysfunction among dengue patients in Islamabad worsened significantly between the 2024 and 2025 epidemic seasons, with mean ALT and AST both rising and a growing minority of patients developing moderate-to-severe transaminase elevation. The divergence between the parametric and non-parametric comparisons of ALT and AST is informative rather than contradictory. The t-

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test, sensitive to the magnitude of extreme values, detected significant mean differences in both ALT ($p = .013$) and AST ($p = .004$). The Mann-Whitney U test, which compares rank distributions and is less influenced by extreme values, found no significant difference (ALT $p = .916$; AST $p = .665$), consistent with the near-identical median ALT (37.0 U/L in both years) and only marginally lower median AST in 2025. Together, these results indicate that the worsening observed at the mean level was not a uniform shift across the whole patient population; rather, it reflects the emergence of a larger subgroup of more severely affected patients in 2025. The "typical" dengue patient in Islamabad had comparable liver enzyme values in both years, while the right tail of the distribution widened substantially.

4.2 NS1 Antigen and Hepatic Injury Mechanisms

The weak 2024 correlation between NS1 level and transaminases is consistent with the expectation that higher viral load is associated with modestly greater hepatic injury, and is directionally consistent with a Kolkata-based report of a stronger NS1 transaminase correlation ($r = .34–.41$) (Palmal et al., 2023); the lower correlation observed here may reflect the predominantly mild disease severity in the 2024 cohort. The loss of this association in 2025 may indicate a shift toward immune-mediated hepatic injury mechanisms plausibly linked to a larger proportion of secondary infections and associated antibody-dependent enhancement in which liver injury becomes less directly tied to circulating antigen level (Halstead, 2007). The markedly wider NS1 range in 2025 (3.14–231.0 vs. 10.5–102.0 in 2024) may also have introduced variability that obscured a linear relationship. In either cohort, NS1 level alone was a poor predictor of hepatic dysfunction severity, reinforcing the case for direct enzyme testing rather than reliance on viral antigen level.

4.3 ALT versus AST: Mechanistic Interpretation

Because AST is present in skeletal muscle, cardiac muscle, and erythrocytes in addition to the liver, the AST-predominant pattern observed in 2025 is consistent with a systemic cytokine-mediated component of tissue injury accompanying hepatocellular damage, rather than hepatic injury alone. This interpretation aligns with Campana et al.'s (2024) systematic review, which reported that AST elevation more frequently exceeded ALT elevation in dengue cohorts and that this pattern was associated with greater disease severity. The very strong ALT–AST correlation observed in both years ($r = .958–.970$) confirms that the two enzymes rise in parallel, while the relatively greater AST rise in 2025 signals a more systemic injury pattern in that season.

4.4 Comparison with Published Studies

Table 7 situates the present findings alongside comparable Pakistani, regional, and international studies. The 2025 Islamabad cohort's overall hepatic dysfunction rate (43.8%) was broadly comparable to, though somewhat lower than, the 53.3% reported by Khan et al. (2025) in Peshawar and the rate implied by Jamil et al.'s (2025) Multan cohort; however, the proportion with moderate AST elevation in the present 2025 cohort (25.0%) exceeded the moderate proportion reported in the Peshawar study (13.3%), while severe involvement was comparatively rare (0.9% vs. 8.9% in Peshawar). This pattern suggests the Islamabad cohorts, drawn from a broader patient base rather than a single tertiary referral center, may capture a milder overall severity spectrum while still showing a clear worsening trend. Sadiq et al.'s (2024) Islamabad pediatric cohort reported AST elevation in 96.7% of patients by day 7 of illness; the present 2025 cohort's higher proportion of pediatric patients (9.8% vs. 1.8% in 2024) may have contributed to its more pronounced AST elevation.

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Table 7. Comparison of present findings with published Pakistani, regional, and international studies.

Study	Setting / year	N	Mean ALT	Mean AST	Hepatic dysfunction	Moderate–severe
Present study (2024)	Islamabad, 2024	112	43.71 U/L	48.99 U/L	42.9%	5 (4.5%) moderate
Present study (2025)	Islamabad, 2025	112	55.55 U/L	72.31 U/L	43.8%	23 (20.5%) moderate
Khan et al. (2025)	Peshawar, 2025	135	~107 U/L	~148 U/L	53.3%	8.9% severe
Jamil et al. (2025)	Multan, 2025	146	~126 U/L	~182 U/L	>64%	AST+ALT >300 linked to mortality
Sadiq et al. (2024)	Islamabad (pediatric), 2024	60	~68 U/L	~140 U/L	68.3% (ALT)	96.7% AST elevated by day 7
Campana et al. (2024)	Global systematic review	167 studies	—	—	14–80%	AST > ALT; predicts mortality
Kalluru et al. (2023)	Global meta-analysis	38 studies	60–120 U/L (moderate)	—	Majority elevated	>200 U/L in severe dengue

4.5 Possible Explanations for the Year-on-Year Worsening

Several converging factors may explain the observed worsening between 2024 and 2025. First, NIH Pakistan's genomic surveillance identified the DENV-2 Cosmopolitan genotype as responsible for 93% of 2024 cases, a serotype associated internationally with greater hepatotropism (NIH Pakistan, 2025). Second, the continuing annual recurrence of dengue epidemics in Pakistan since 2005 has likely increased the pool of previously infected individuals at risk of secondary infection, raising the potential contribution of antibody-dependent enhancement to hepatic injury (Halstead, 2007). Third, the broader age spectrum observed in the 2025 cohort, with more pediatric and elderly patients, may have contributed to greater hepatic vulnerability, as both age groups are recognized as having reduced hepatic reserve. Fourth, the escalating overall epidemic burden in Pakistan, from 3,442 cases in 2020 to more than 20,000 in 2024, raises the possibility that a larger and more diverse pool of presenting patients, including more severe cases, was captured in the 2025 sample.

4.6 Clinical Implications

These findings carry several practical implications for dengue case management in Islamabad. The high proportion of moderate hepatic dysfunction in 2025 (20.5% by ALT; 25.0% by AST), likely to be largely asymptomatic, supports routine liver function testing at the point of dengue diagnosis rather than testing reserved for patients with overt signs such as jaundice or hepatomegaly, a recommendation also made by Khan et al. (2025) and Jamil et al. (2025). The emergence of a severe AST case in 2025, where none occurred in 2024, underscores the value of early biochemical monitoring to permit timely referral. At the same time, the stable median values across years indicate that most dengue patients in Islamabad can continue to be managed at the primary care level, provided clinicians remain alert to the growing minority who require closer monitoring or referral.

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4.7 Limitations

This study has several limitations. Analysis was restricted to five variables (NS1, age, gender, ALT, AST); other clinically relevant measures such as platelet count, bilirubin, albumin, INR, length of stay, and mortality were not available. As a retrospective study, findings depend on the accuracy of existing records, and although a systematic quality control review was performed, some inter-site variability in laboratory analyzers cannot be excluded. The precise timing of specimen collection relative to illness onset in the 2025 cohort was not documented beyond the epidemic season, which may affect comparability given that hepatic involvement typically peaks around days 3–7 of illness. The identity of the 2025 source hospital could not be disclosed under the data-sharing agreement, and its referral profile may differ from that underlying the 2024 dataset, introducing potential selection bias. Individual dengue serotyping was not available, precluding patient-level analysis of serotype-specific hepatic injury. Finally, as a Bachelor of Science-level research project involving 112 patients per cohort, this study should be regarded as a focused, hypothesis-generating analysis rather than a definitive population-level clinical trial, and its findings warrant confirmation in larger, prospective, multicenter studies before informing clinical guidelines.

5. Conclusion

This comparative cohort study found that hepatic dysfunction among dengue patients in Islamabad worsened significantly between the 2024 and 2025 epidemic seasons. Mean ALT rose by 27.1% ($p = .013$) and mean AST by 47.6% ($p = .004$); moderate ALT elevation increased more than fourfold (4.5% to 20.5%, $p = .001$) and moderate AST elevation doubled (12.5% to 25.0%, $p = .009$), with the first recorded severe AST case appearing in 2025. These changes justify rejection of the null hypothesis in favor of the alternative hypothesis of a significant year-on-year shift in hepatic involvement. Notably, the non-significant Mann-Whitney U results and stable median values indicate that this worsening was concentrated in a growing subgroup of more severely affected patients rather than reflecting a shift across the entire patient population. NS1 antigen level correlated weakly with liver enzymes in 2024 but not in 2025, suggesting a possible shift toward immune-mediated hepatic injury mechanisms, while gender distribution remained comparable across years and the 2025 cohort included a broader age range.

These findings support several practical recommendations, limited to those already grounded in the study's data. Clinicians managing dengue patients in Islamabad should consider routine ALT and AST testing at diagnosis, given that a substantial proportion of moderate hepatic dysfunction is likely to be clinically silent; patients with moderate elevation warrant monitoring, and those with severe elevation warrant prompt referral. Pediatric and elderly dengue patients, over-represented among the more severely affected group in 2025, may benefit from closer hepatic monitoring. At the policy level, these results add Islamabad-specific, year-on-year evidence to the broader case for incorporating routine liver function testing into national dengue management guidance. Future research building on this work should incorporate a wider set of clinical variables, individual-level serotyping, and serial (rather than single time-point) enzyme measurements, ideally in a prospective multicenter design, to clarify whether the worsening trend identified here continues in subsequent dengue seasons.

This study contributes the first comparative, year-on-year analysis of dengue-associated hepatic dysfunction from Islamabad, using a consistent battery of statistical tests across two annual cohorts, and provides quantitative, locally derived evidence that can inform both clinical vigilance and future policy discussion regarding hepatic monitoring in Pakistan's national dengue response.

Author Contributions

Complete using a standard taxonomy such as Credit, e.g.: Muhammad Zaheer.: conceptualization, data curation, formal analysis, writing original draft; Said Ahmad Shah.: data curation, investigation ,writing review & editing;

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Tofeeq Ur Rahman.: data curation, investigation; Hanzala Basharat: formal analysis, writing review & editing; Ghulam Abbas.: investigation, writing – review & editing; Tanveer Tara.: supervision, conceptualization, writing review & editing. All authors read and approved the final manuscript.

Data Availability

The 2024 dataset generated and analyzed during the current study is available from the corresponding author on reasonable request. The 2025 dataset cannot be made publicly available because it was obtained from the collaborating hospital under a formal data-sharing and confidentiality agreement that prohibits redistribution and does not permit disclosure of the source institution; requests regarding this subset should be directed to the corresponding author, who will advise on the applicable access constraints.

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