

Correlation of Liver Function Test and Severity in Dengue PatientsSaradva B¹, Gupta P², Shrimali L³, Shah M⁴¹3rd Year Resident, Department of Medicine, Geetanjali Medical College and Hospital, Rajasthan²Associate Professor, Department of Gastroenterology, Geetanjali Medical College and Hospital, Rajasthan³Professor, Department of Medicine, Geetanjali Medical College and Hospital, Rajasthan⁴Senior Resident, Department of Dermatology, Geetanjali Medical College and Hospital, Rajasthan

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Abstract:**Introduction:** Dengue Viral infection (caused by Flaviviridae group) has recently seen a spurt affecting multiorgan. Most of the cases are asymptomatic, while few patients present with fulminant symptoms.**Aim:** To assess association of liver function test and severity in Dengue.**Material and Methods:** This prospective observational study was conducted in Geetanjali Medical College at tertiary care hospital of southern Rajasthan from June 2020 to November 2021 after institutional ethics committee approval. In this study, all dengue positive cases based on positive ELISA test, NS1 antigen, IgM and IgG were included and cases diagnosed with malaria, liver cirrhosis, viral hepatitis, enteric fever and patients taking hepatotoxic drugs were excluded. Diseases which can derange Liver function test (LFT) were also excluded. Liver function was evaluated for all patients and its association with dengue severity and patient's clinical outcomes were assessed.**Results:** This study showed that serum bilirubin levels were elevated in dengue fever patients. Out of 157 patients, mean total bilirubin of the patients was 1.56 ± 0.17 , 1.56 ± 0.16 and 3.59 ± 0.83 mg/dl in the DF (Dengue fever), DHF (dengue hemorrhagic fever) and DSS (dengue shock syndrome) group respectively. Total of 140 patients (91.5%) had raised AST (Aspartate transaminase) levels (> 40 U/L). DF group had 89 patients (87.3%) with raised AST (mean AST 154.17 ± 44.73), where all patients of DHF 37 patients (100%) (mean AST 355.87 ± 8.26) and DSS 14 patients (100%) (mean AST $814.79.17 \pm 36.65$) had raised AST level (p value < 0.01). Likewise, 137 patients (89.5%) had raised ALT (Alanine transaminase) levels (> 40 U/L). DF group had 86 patients (84.3%) (Mean AST 91.7 ± 22.41) with raised ALT, where all patients of DHF 37 patients (100%) (Mean AST 136.6 ± 6.76) and DSS 14 patients (100%) (Mean AST 368.86 ± 13.63) had raised AST level.**Conclusion:** We conclude the rising levels of AST and ALT are associated with DHF and DSS severity and hence a baseline liver function test should be done in every dengue patient.**Keywords:** Dengue Fever, Liver Function Test, Dengue Hemorrhagic Fever, Dengue Septic Shock.This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided original work is properly credited.**Introduction**

Dengue fever's worldwide burden has more than quadrupled in the previous three decades, and there are currently 2.5 billion individuals at risk of the illness. [1] Dengue virus infections may produce a broad range of clinical symptoms, from a moderate febrile sickness known as 'dengue fever' to 'severe dengue,' also known as dengue hemorrhagic fever (DHF). [2,3,4] The median aspartate transaminase (AST) and alanine transaminase (ALT) levels for severe dengue fever were reported to be greater than for simple dengue fever [5] Raised aspartate transaminase (AST) values are observed in around 63–97% of patients, while high alanine transaminase (ALT) levels are seen in 45–96% of patients. This suggests a probable link between elevated transaminase levels and disease

severity.[6] Changes in the liver function test may serve as an early marker for timely diagnosis and identification of patients who may develop severe complications.[5] The goal of this research was to assess the relationship between Liver function test (LFTs) and early diagnosis and severity of Dengue fever, as well as the relationship between complications, hospital stay, and ultimate result (survival/mortality) and illness severity.

Material and Methods

This prospective observational study was conducted in Geetanjali Medical College at tertiary care hospital of southern Rajasthan. It was conducted from June 2020 to November 2021. The approval of the institutional ethics committee for

our study was obtained (GU/HREC/EC/2019/1720). In this study, all dengue positive cases based on positive ELISA test, NS1 antigen, IgM and IgG were included and cases diagnosed with malaria, liver cirrhosis, viral hepatitis, enteric fever and patients taking hepatotoxic drugs were excluded. Diseases which can derange LFT were also excluded. Liver function was evaluated for all patients and its association with dengue severity and patients' clinical outcomes were assessed.

Data Collection

Data were collected using a pre-designed semi-structured study proforma. Demographic information, Clinical information and findings of the general physical and systemic examination were noted. Findings of various laboratory investigations like complete blood count (CBC), liver function test (LFT), Hepatitis B surface antigen (HBsAg), NS1 Early dengue ELISA, Commercial capture IgG & IgM were also noted for all patients.

Operational Definitions [7]

1. Diagnosis of dengue infection: Febrile illness associated with detection of dengue specific IgM capture antibody or NS1 antigen.
2. Dengue Hemorrhagic Fever (DHF): patients who have DF and hemorrhagic manifestations, low platelet count, and objective evidence of leaky capillaries ($\geq 20\%$ elevation in hematocrit, lower serum albumin, and pleural or other effusions) were classified as having DHF (WHO grades I/II)
3. Dengue Shock Syndrome (DSS): Those with evidence of circulatory failure (pulse pressure ≤ 20 mmHg, hypotension, or frank shock) were classified as having DSS (WHO classification, DHF grades III/IV).

DHF and DSS were classified as severe dengue infection.

Statistical Analysis

The analysis included profiling of patients on different demographic, laboratory and clinical parameters. Descriptive analysis of quantitative parameters was expressed as means and standard deviation. Ordinal data were expressed as absolute number and percentage. Cross tables were generated and chi square test was used for testing of associations and Student t test was used for comparison of quantitative parameters between DF, DHF and DSS group of patients. P-value < 0.05 is considered statistically significant. All analysis was done using SPSS software, version 24.0.

Result

The study enrolled 153 patients who met the study requirements, 101 (66.01 %) of whom were male

and 52 (33.98%) were female. Among them, 102 (66.7 %) had DF, 37 (24.2 %) had DHF, and 14 (9.2 %) had DSS (table 1)

In this study, the most common presenting complaint was fever in 76 (49.6%) followed by pain abdomen in 70 patients (45.7%), rash in 62 (40.5%) patients, vomiting in 62 (39.2%) patients, headache in 54 (35.2%) patients and joint pain in 51 (33.3%) patients. It was also observed that bleeding was found in 44 patients (28.75%) out of 153 patients. (Table 2)

Mean hemoglobin of the patients was significantly higher in DSS group of patients, as compared to those in the DF and DHF group of patients, and mean hematocrit of the patients was 43.67 ± 2.03 , 44.14 ± 1.31 and 47.21 ± 2.63 percent in the DF, DHF and DSS group respectively. However, mean platelet count of the patients was significantly lower in DSS (48358.79 ± 27786.69 per ml) group as compared to those in the DF group of patients (87775.24 ± 14374.31 per ml) and DHF group of patients (75785.03 ± 26761.81 per ml) (Table 3)

Out of 157 patients, mean total bilirubin of the patients was 1.56 ± 0.17 , 1.56 ± 0.16 and 3.59 ± 0.83 mg/dl in the DF, DHF and DSS group respectively. Mean direct bilirubin of the patients was 0.48 ± 0.06 , 0.48 ± 0.06 and 1.83 ± 0.73 mg/dl in the DF, DHF and DSS group respectively. (Table 4). It was observed that mean total protein of the patients was 6.24 ± 0.49 , 6.26 ± 0.57 and 6.19 ± 0.31 gm/dl in the DF, DHF and DSS group respectively. Mean serum albumin of the patients was 3.46 ± 0.32 , 3.44 ± 0.31 and 3.51 ± 0.34 gm/dl in the DF, DHF and DSS group respectively. 140 patients (91.5%) had raised AST levels (> 40 U/L). DF group had 89 patients (87.3%) with raised AST (mean AST 154.17 ± 44.73), where all patients of DHF 37 patients (100%) (mean AST 355.87 ± 8.26) and DSS 14 patients (100%) (mean AST $814.79.17 \pm 36.65$) had raised AST level (p value < 0.01). Likewise, 137 patients (89.5%) had raised ALT levels (> 40 U/L). DF group had 86 patients (84.3%) (mean AST 91.7 ± 22.41) with raised ALT, where all patients of DHF 37 patients (100%) (mean AST 136.6 ± 6.76) and DSS 14 patients (100%) (mean AST 368.86 ± 13.63) had raised AST level (p value < 0.01) (Table 5).

Discussion

In over 100 countries, an estimated 99 million (95 percent credible range 71–137 million) symptomatic dengue infections and 404 million asymptomatic (95 percent credible interval 304–537 million) infections occur each year, with 50,000 severe cases and 20,000 fatalities. Early detection of prognostic markers to assess its severity is the mainly effective approach to control these fatalities. [4,8,9] In this study a significant relationship observed between rising age and female sex with increased dengue severity (p value

0.01). Comparable research done by Bandopadhyaya et al [10] & Soni et al. [11] Though Rajoo Singh et

al [12] enrolled 214 participants in a comparable trial.

Table 1: Association of age with severity of dengue

Age groups (years)		Dengue severity			Total
		DF	DHF	DSS	
Less than 20	N	12	0	0	12
	%	11.80%	0.00%	0.00%	7.80%
21 to 40	N	54	5	0	59
	%	52.90%	13.50%	0.00%	38.60%
41 to 60	N	28	20	6	54
	%	27.50%	54.10%	42.90%	35.30%
61 to 80	N	8	12	8	28
	%	7.80%	32.40%	57.10%	18.30%
Total	N	102	37	14	153
	%	100.00%	100.00%	100.00%	100.00%
		p value* < 0.01			

*analyzed using chi-square test

Table 2: Association of clinical findings with severity of dengue

Clinical findings		Dengue severity			Total	p value*
		DF	DHF	DSS		
Bleeding	N	0	35	9	44	< 0.01
	%	0.00%	94.60%	64.30%	28.80%	
Shock	N	2	4	14	20	< 0.05
	%	2.00%	10.80%	100.00%	13.10%	
Hematuria	N	7	2	2	11	0.53
	%	6.90%	5.40%	14.30%	7.20%	
Total	N	102	37	14	153	
	%	100.00%	100.00%	100.00%	100.00%	

*analyzed using chi-square test

Table 3: Comparison of hematological parameters between patients with DF, DHF and DSS

Hematological investigations	DF		DHF		DSS		P value*
	Mean	SD	Mean	SD	Mean	SD	
Hematocrit(%)	43.67	2.03	44.14	1.31	47.21	2.63	< 0.05
Hemoglobin(gm%)	11.65	2.08	12.46	2.28	14.79	2.61	< 0.01
Platelet (per ml)	87775.24	14374.31	75785.03	26761.81	48358.79	27786.69	< 0.01
Total Leucocyte Count (per cu mm)	10460.75	2324.17	12098.78	3705.43	15270.07	3316.65	< 0.05
Prothrombin time (seconds)	12.49	2.50	12.51	4.51	12.43	3.51	0.86

*analyzed using ANOVA test

Table 4: Comparison of liver function parameters between patients with DF, DHF and DSS

Bilirubin	DF		DHF		DSS		p value*
	Mean	SD	Mean	SD	Mean	SD	
Total bilirubin(mg/dl)	1.56	0.17	1.56	0.16	3.59	0.83	< 0.05
Direct bilirubin(mg/dl)	0.48	0.06	0.48	0.06	1.83	0.73	< 0.05

*analyzed using ANOVA test

Table 5: Comparison of serum protein levels, serum liver enzyme levels, raised AST between patients with DF, DHF and DSS.

Serum protein	DF		DHF		DSS		p value*
	Mean	SD	Mean	SD	Mean	SD	
Total protein(gm/dl)	6.24	0.49	6.26	0.57	6.19	0.31	0.88
Albumin(gm/dl)	3.46	0.32	3.44	0.31	3.51	0.34	0.75
Globulin(gm/dl)	2.77	0.60	2.82	0.62	2.67	0.44	0.71
Liver enzymes							
Aspartate aminotransferase[AST] (U/L)	154.17	44.73	355.87	8.26	814.79	36.65	< 0.01
Alanine Aminotransferase[ALT] (U/L)	91.77	22.41	136.60	6.76	368.86	13.63	< 0.01

Alkaline phosphatase[ALP] (U/L)	135.70	17.86	133.30	19.02	136.29	17.31	0.76
		Dengue severity				Total	
Raised AST (> 40 U/L)		DF	DHF	DSS			
Yes	N	89	37	14		140	
	%	87.30%	100.00%	100.00%		91.50%	
No	N	13	0	0		13	
	%	12.70%	0.00%	0.00%		8.50%	
Total	N	102	37	14		153	
	%	100.00%	100.00%	100.00%		100.00%	
		p value* < 0.01					

In which preponderance of male (76.6%) versus female (23.4%) were observed. In those individuals, 174 (81.3 percent) were diagnosed with dengue fever, 29 (13.6 percent) with dengue hemorrhagic fever, and 11 (5.1%) with dengue shock syndrome. In their study, the average age of the patients was 31.6 years. Dengue fever is ubiquitous in tropical and subtropical regions across the globe, mostly in urban and semi-urban settings. Minor discrepancies in the distribution of patients based on severity might be attributed to their geographic location. In the preceding studies, [10,11,12,13,14] fever was the most commonly found symptom in dengue fever, which simulated this study; however, it was observed that fever was found in all patients before admission or at the time of admission, whereas in this study, only presented symptoms were included at the time of admission.

Typically, the disease begins with a sudden onset of fever with severe headache and any of the following symptoms: chills, pain behind the eyes, particularly on eye movement, backache, and pain in the muscles, bones, and joints pain. In this research, 44 individuals were found to have bleeding. Bleeding was more prevalent in DHF (94.6%) followed by DSS (64.3%) and DF group (0 percent). Patients with bleeding were shown to be more prevalent in those who had a high AST, a rising hematocrit, a lower platelet count, a lower PT and a longer aPTT, and a lower fibrinogen level during the fever. Earlier research [10,13,15] found the majority of the symptoms of dengue infection were identical to this finding.

In this study the mean hemoglobin of the patients was significantly higher in the DSS group (14.7 ± 2.61 gm/dl) than in the DF (11.6 ± 2.08 gm/dl) and DHF (12.4 ± 2.2 gm/dl) groups, p value 0.01 which is statistically very significant. It was found that DSS, DHF, and DF boosted hematocrit levels while decreasing platelet count in dengue patients. Rajoo Singh et al found that the average haemoglobin was 13.8 ± 0.17 gm/dl, the hematocrit was 40.6 ± 0.5 percent, the total leukocyte count was 6123 ± 339 cells/mm³, and the platelet count was $48.5 \pm 2.6 \times 1000$ /mm³. In the research by Sanjay T Patil et al, 12 patients (12%) were anemic (Hb percent 10gm/dl), 30 patients (30%) had increased hematocrit, 55 patients (55%) had leucopenia (TC 4000 cells/cu.mm), and 84 percent of patients had

thrombocytopenia (platelet count 1.5 lakhs/cu.mm). Kittittrakul et al [13] found that the mean hematocrit (percent) in their research was 42.75 ± 5.19 , the mean TLC (cells/cu.mm) was $3,983.45 \pm 1,925.08$, and the mean Platelets (lakhs/cu.mm) was $61905.95 \pm 39561.33.58$. It was found that DSS, DHF, and DF boosted hematocrit levels while decreasing platelet count in dengue patients. An increase in hematocrit indicates that plasma leakage occurred as a result of widespread capillary leak, hypotension, rhabdomyolysis, hemolysis, and severe DIC, resulting in ischemia, hypoxia, and multiorgan failure, and that correct fluid management is necessary. [16,17]

This study showed that serum bilirubin levels were elevated in dengue fever patients which simulates with above studies. [10,11,18] Though the mean total protein, mean serum albumin, mean serum globulin level was not significantly raised in DF, DHF and DSS (P VALUE >0.05) which were also support the previous studies.

Amit Soni et colleagues found that out of 281 individuals, 277 (93.6 percent) had elevated AST levels and 270 had elevated ALT level.[11] In Rajoo Singh's research, [12] 209 patients (97.7 percent) had elevated AST levels, with 169 patients (97.1 percent) having DF, 29 patients (100 percent) having DHF, and 11 patients (100 percent) having DSS. ALT levels were elevated in 199 patients (93.9%), with 161 patients (92.5%) having DF, 27 patients (93.1%) having DHF, and 11 patients (100%) having DSS. Above studies simulates with our study. AST and ALT levels were considerably elevated in dengue patients. AST is present in erythrocytes, cardiac and skeletal muscle, kidney, liver, and brain tissue, while ALT is mostly found in the liver. This pattern is present in alcoholic hepatitis and is reversed in viral hepatitis.

The reasons for the change in liver function tests in Dengue patients include increased infection of hepatocytes, which leads to apoptosis, and high levels of endoplasmic reticulum stress-driven apoptosis, which is one example of dengue induced hepatocyte apoptosis.[7] The transcription factor NF-B has also been linked to the induction of apoptosis. The antibody-dependent enhancement hypothesis for the mechanism of DHF/DSS development explains the increased risk for DHF/DSS in patients who had a second dengue

infection of a different serotype from a previous infection; residual antibody from a previous infection may bind to the virus of the second episode and construct the virus-anti-body complexes.[11] These complexes are more readily taken up by cells expressing Fc receptors, such as monocytes and macrophages, than uncoated virus. Following this, a series of cytokine cascades will occur, followed by complement activation and an increase in endothelial and vascular permeability. Cytokines implicated in the pathophysiology of DHF/DSS include interferon, TNF, IL-2, IL-6, IL-1, and IL-8,10,11,12

Limitations: This was a single hospital-based study with limited sample size and duration. More multi-centric research with a bigger sample size is needed to back up our results.

Conclusion

Based on the results of our study, we conclude that raised AST and ALT levels were significantly associated with severity of DSS and DHF. We recommend that patients with dengue infection should have a baseline liver function test (LFT) and its monitoring to identify early hepatic damage. Future research should look at the patient risk factors for hepatic impairment in dengue infection.

Declaration of Patient Consent: The authors certify that they have obtained all appropriate patient consent forms.

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