RESEARCH ARTICLE

Evaluation of Serum Lipid Profile Changes as Diagnostic and Prognostic Biomarkers in Paediatric Dengue Infection across WHO Disease Severity Classes: A Comparative Observational Study

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ABSTRACT

Background: Dengue fever is a serious illness caused by a virus that can lead to major health issues and even death in children who live in tropical regions. It is still hard to tell which children are likely to get very sick early on. This study examined how blood fat levels, including triglycerides, good cholesterol, bad cholesterol, and total cholesterol, changed based on different levels of dengue severity according to the World Health Organization. It also looked into whether these changes could help predict how serious the dengue illness was and how long a patient might need to stay in the hospital.

Methods: A comparison study was done over 18 months in the Paediatric Ward and PICU of a tertiary care centre at Puducherry, India. Sixty kids with confirmed dengue cases, aged between 1 and 14, were part of the study. They were split into three groups, each with 20 people, according to the WHO (2009) guidelines: Dengue Without Warning Signs, Dengue With Warning Signs, and Severe Dengue. When the patient came in, their blood lipid levels and blood test results were tested. The study used statistical techniques such as One-Way ANOVA, Pearson correlation, and multivariable linear regression to examine the data.

Results: All four lipid markers showed substantial variations between severity groups, according to the study ($p<0.0001$). HDL had the biggest progressive drop and the strongest association with the severity of the illness. TC and LDL showed progressive, parallel decreases. TG exhibited an inverse elevation pattern, peaking in DWWS and falling in SD. An HDL threshold of less than 25 mg/dL yielded 85.0% sensitivity and 96.3% specificity for severe dengue; the combined lipid panel accounted for 64.1% of the variation in hospital stay. Low HDL and thrombocytopenia were 100% co-occurring in severe dengue cases.

Conclusion: There is a strong correlation between increases in blood lipid levels and the severity and duration of paediatric dengue. HDL cholesterol is a highly sensitive and specific biomarker for risk assessment in resource-constrained tropical environments.

Keywords: Dengue, Pediatrics, Lipids, High-Density Lipoprotein, Biomarkers, Disease Severity, Hospital Stay

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INTRODUCTION

The dengue virus, often known as DENV, is a type of single-stranded RNA virus that causes dengue fever. There are four distinct serotypes (numbers 1 through 4) of this virus.It is a member of the Flaviviridae family of viruses. *Aedes aegypti* and *Aedes albopictus* are the two mosquito species that transmit the virus [1, 2]. In tropical and subtropical regions, it is a serious health risk. According to estimates of the global burden, approximately 390 million infections occur year, and in regions where the disease is prevalent, children are primarily impacted by severe illness [3,4]. The healthcare system is under a lot of strain because India accounts for a significant portion of all cases worldwide and frequently has outbreaks during specific seasons [5,6].

Dengue can cause a wide variety of symptoms in children, from fever without obvious symptoms to severe cases with numerous organ damage, severe bleeding, and fluid leakage from blood vessels [7, 8, 9]. To aid in better triage and early treatment, the WHO 2009 categorization divides patients into three groups: Dengue Without Warning Signs (DWWS), Dengue With Warning Signs (DWS), and Severe Dengue (SD) [7, 10]. Antibody-dependent enhancement is how the immune system responds to a second infection with a different virus. This can cause a severe reaction in which the body releases a lot of certain proteins, such as TNF-α, IL-6, and IFN-γ, which can damage and cause small leakage in blood vessel cells [11-14].

There are few early indicators that can indicate a bad outcome prior to plasma leakage, even though standard tests like hematocrit and platelet count aid in tracking the disease's course [8]. Recently, abrupt viral infections have brought attention to issues with the body's fat metabolism [15]. Cell membranes, steroid signalling, and the complexes that facilitate viral entry and replication all depend on lipids [16,17]. The body releases specific chemicals known as cytokines in response to a severe viral infection. These chemicals alter the function of an enzyme called lipoprotein lipase, slow down the liver's generation of fats, and accelerate the clearance of fat particles from the blood.[18,19].

The present study aims to examine the changes in blood lipid levels in children with various WHO-classified dengue fever levels, determine how these lipid levels relate to the severity of the illness and the length of the child's hospital stay, and determine whether these lipid levels can be used as markers to aid in medical decision-making [20].

MATERIALS AND METHODS

A comparative observational study was conducted over an 18-month period in the Paediatric Ward and Pediatric Intensive Care Unit (PICU) in a tertiary care centre at Puducherry after approval from Institutional Ethics Committee (Ref no: 23/SVMCH/IEC-Cert/February24). After purposive sampling 60 paediatric children aged 1 to 14 years with serologically confirmed dengue infection were chosen for the study after obtaining written parental/guardian consent and child assent. Serologically confirmed dengue infection (NS1 antigen positive and/or IgM ELISA positive) in children aged 1–14 years were included in the study. IgM non-reactive dengue-like illness; pre-existing liver disease; obesity according to Indian Academy of Paediatrics (IAP) growth charts (for ages 5–14 years) or WHO growth charts (for ages 1–4 years); underlying metabolic disorders; or acute bacterial co-infections were excluded from the study.

Classification and Patient Management

Patients were assigned to three equal cohorts (n=20 per group) based on the WHO (2009) criteria:

1. **Group 1 (DWWS):** Dengue Without Warning Signs
2. **Group 2 (DWS):** Dengue With Warning Signs
3. **Group 3 (Severe Dengue):** Severe Dengue (severe plasma leakage leading to shock or fluid accumulation with respiratory distress, severe bleeding, or severe organ impairment)

LABORATORY INVESTIGATIONS

Following an 8–12 hour fast, venous blood samples were obtained upon entry. IgM capture ELISA and NS1 antigen were used for serological confirmation. Automated hematology analyzers were used to measure complete blood counts (CBCs). Automated enzymatic clinical chemistry analyzers were used to measure the parameters of the fasting serum lipid profile, including total cholesterol (TC), HDL cholesterol, LDL cholesterol, and serum triglycerides (TG).

The sample size was determined using Epi Info (v3.0.1) based on literature data (Siddiqui et al.) reporting mean TG levels of 240.23 mg/dL (DWWS), 191.67 mg/dL (DWS), and 165.50 mg/dL (SD) [21]. A minimum of 20 patients each group (N=60 total) were needed, with an 80% statistical power, a 5% alpha level, and a 10% acceptable error.

STATISTICAL ANALYSIS

Microsoft Excel 2010 was used to manage the data, and SPSS version 23.0 was used for analysis. The mean ± standard deviation (SD) and median (interquartile range/range) were used to express continuous variables. Frequencies and percentages were used to display categorical variables. One-Way Analysis of Variance (ANOVA) for continuous variables and Pearson's Chi-square test ($\chi 2$) for categorical variables were used to evaluate differences across the three severity groups. Independent sample t-tests were used for post-hoc pairwise comparisons. Pearson's correlation coefficient (r) was used to assess correlations between lipid markers, dengue severity score (coded 1–3), and duration of hospital stay. To find independent predictors of hospital stay, simple and multiple linear regression analyzes were carried out. Statistical significance was defined as a two-tailed p-value of less than 0.05.

RESULTS

The results of the study were presented in tables 1-8.

Sixty children with dengue were included in the study; their average age was 7.63 years, plus or minus 3.06 years. 43.3% of the patients were between the ages of 6 and 10. 55.0% of the population was male (M:F=1.22:1). In all three severity levels, the age and gender groups were sufficiently similar ($p>0.05$), indicating that the groups' composition was essentially the same (Table 1 and Table 2). 100% of the 60 patients had dengue,

according to serological testing. Overall, 40 out of 60 samples (66.7% of the tests) tested positive for the NS1 antigen, while 42 out of 60 samples (70.0%) tested positive for IgM ELISA.

Clinical Features and Physical Signs

Every single patient had a fever, from 5% in DWWS to 90% in Severe Dengue, abdominal discomfort showed the highest correlation with illness severity ($\chi 2=29.77$, $p<0.0001$) (Table 4). Additionally, there was a statistically significant increase in vomiting ($p=0.0004$), mucosal bleeding ($p=0.0001$), edema ($p=0.0004$), and skin rash ($p=0.029$) with the severity of the condition. Hepatomegaly increased gradually from 10% in DWWS to 85% in Severe Dengue ($p<0.0001$).

Hematological Parameters

The platelet count fell sharply throughout severity groups ($F=83.20$, $p<0.0001$), from 234.60±72.59×103/μL in DWWS to 42.70±22.56×103/μL in Severe Dengue (Table 6). Thrombocytopenia (<100×103/μL) was present in all severe cases. Furthermore, a substantially lower total leucocyte count (TLC) was linked to severe sickness ($F=4.26$, $p=0.019$). There was no significant difference between DWWS and DWS, according to post-hoc analysis ($p=0.611$).

Serum Lipid Profile Dynamics

Significant alterations were documented across all lipid fractions:

- **HDL Cholesterol:** HDL was found to be more ($F=103.58$, $p<0.0001$). Mean HDL was 44.10±6.36 mg/dL in DWWS, 28.00±5.51 mg/dL in DWS, and 19.35±4.55 mg/dL in Severe Dengue (>55% total reduction). Hypoalphalipoproteinemia (<40 mg/dL) was present in 100% of DWS and Severe Dengue patients.

- **Total Cholesterol:** TC decreased from 158.40±19.66 mg/dL in DWWS to 127.10±21.68 mg/dL in DWS and 96.00±13.03 mg/dL in Severe Dengue ($F=56.91$, $p<0.0001$). All severe dengue cases (100%) had hypocholesterolemia (<140 mg/dL).

- **LDL Cholesterol:** LDL levels dropped from 86.50±18.71 mg/dL in DWWS to 65.25±12.81 mg/dL in DWS and 47.75±10.64 mg/dL in Severe Dengue ($F=36.02$, $p<0.0001$). Low LDL (<70 mg/dL) was observed in 100% of severe cases.

Length of Hospital Stay

The average length of hospital stay rose with the severity of the disease: 4.00±0.86 days in DWWS, 6.10±1.33 days in DWS, and 9.05±1.00 days in Severe Dengue ($F=109.92$, $p<0.0001$). The length of hospital stay and the dengue severity score showed significant inverse relationships with all lipid measures (Tables 16 and 17). The highest link between disease severity and HDL cholesterol ($r=-0.872$, $r2=0.760$, $p<0.0001$) was followed by total cholesterol ($r=-0.816$), platelet count ($r=-0.842$), LDL ($r=-0.746$), and TG ($r=-0.701$). Hospital stay ($F = 109.92$) and HDL ($F = 103.58$) were the two most discriminating indicators when all parameters were ranked by ANOVA F-statistic, followed by platelet count ($F = 83.20$), total cholesterol ($F = 56.91$), LDL ($F = 36.02$), and triglycerides ($F = 29.24$). The least effective discriminator was TLC ($F = 4.26$). All lipid parameters had large effect sizes ($\eta^2 = 0.50–0.78$). These results show that the serum lipid profile, as a panel, offers discriminatory information that is on par with or better than established hematological markers in pediatric dengue and should be regarded as a routine investigation in all confirmed pediatric dengue admissions to direct early severity assessment, clinical monitoring, and resource allocation decisions.

Hospitalization stay was the most objective clinical outcome measure, as evidenced by its progressive increase with dengue severity: DWWS 4.00 ± 0.86 days, DWS 6.10 ± 1.33 days, and severe dengue 9.05 ± 1.00 days ($F = 109.92$, $p < 0.0001$). Pairwise differences were all quite substantial. HDL ($R^2 = 0.573$), platelet count ($R^2 = 0.572$), and LDL ($R^2 = 0.568$) each explained almost 57% of the variance in hospital stays in simple linear regression. 64.1% of the variance in hospital stays was explained by the combined lipid panel in multiple regression (Adjusted $R^2 = 0.616$, $F = 24.56$, $p < 0.0001$). In the multivariable model, triglycerides lost individual significance ($p = 0.077$), indicating shared variance with other lipid measures. This result is in keeping with the collinear biological processes influencing all dengue lipoprotein fractions. All lipid metrics significantly distinguished between each pair of severity groups ($p<0.01$), according to post-hoc pairwise comparisons. Composite marker evaluation revealed that 0% of DWWS, 45% of DWS, and 100% of Severe Dengue cases had both thrombocytopenia (<100×103/μL) and low HDL (<40 mg/dL) at the same time ($p<0.0001$).

Clinical utility cut-off thresholds were determined. An admission HDL threshold of <40 mg/dL gave 100.0% sensitivity for recognizing cases beyond mild dengue, according to a diagnostic accuracy analysis for HDL cholesterol. For the detection of severe dengue, a threshold of HDL <25 mg/dL yielded 85.0% sensitivity, 96.3% specificity, a positive predictive value (PPV) of 94.4%, and an overall diagnostic accuracy of 91.7%.

DISCUSSION

The current study assessed changes in serum lipid levels in pediatric patients across WHO dengue severity classes. The results demonstrate that dengue virus infection significantly disrupts serum lipid homeostasis and the degree of these changes is highly correlated with hospital stay and clinical severity.

The majority of the children included were aged 6-10 years (43.3%), with a little excess of males (55%), which

is consistent with paediatric dengue epidemiological trends in South Asia. Male dominance is typically associated with increased outside daylight activities, which leads to greater exposure to vectors. The demographic parameters were consistent across severity groups ($p > 0.05$), showing that age and gender are not confounding variables. Severe illness is strongly associated with symptoms including abdominal pain ($\chi 2=29.77$) and hepatomegaly (seen in 85% of severe cases), supporting their importance as clinical markers of the disease.

Serum TG levels increased initially in mild disease (240.65±34.95 mg/dL in DWWS) and decreased in severe cases (165.40±24.18 mg/dL). As the disease progresses, hepatic dysfunction limits VLDL synthesis, and viral membrane assembly consumes TG-rich particles, resulting in a relative fall in TG levels. These findings are consistent with those reported by Siddiqui et al. and Limo et al., who showed comparable TG patterns in acute dengue [21,22]. The lipid changes reported in this study are consistent with regional and international literature. The biggest lipid discriminator was HDL cholesterol ($F=103.58$, $r=-0.872$), which is consistent with research by Palma et al. and Suvarna et al., who identified reduction of protective HDL as a sign of systemic inflammation and vascular leakage [23,24]

CONCLUSION

Serum lipid markers (TG, HDL, LDL, and Total Cholesterol) show significant changes in pediatric dengue infection, which correlates with WHO disease severity classes and length of hospital stay. HDL cholesterol had the highest connection with disease severity ($r=-0.872$, $p<0.0001$), and an admission cut-off of <25 mg/dL provided good diagnostic accuracy for severe dengue. The multivariable combined lipid panel explained 64.1% of the variation in length of hospital stay. Fasting lipid profile, together with conventional hematological tests, provides an accessible, objective technique for early risk classification in pediatric dengue.

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TABLES AND FIGURES

Table 1: Age And Sex Distribution Across Dengue Severity Groups

Variable	DWWS (n=20)	DWS (n=20)	Severe (n=20)	Total (N=60)	p-value
Mean Age ± SD (years)	7.20±3.00	8.05±3.07	7.65±3.20	7.63±3.06	0.611
Median Age (years)	6.50	7.50	8.50	7.50	—
Age Range (years)	3 – 14	2 – 13	2 – 14	2 – 14	—
Male, n (%)	11 (55%)	12 (60%)	10 (50%)	33 (55%)	0.797
Female, n (%)	9 (45%)	8 (40%)	10 (50%)	27 (45%)	—
M:F Ratio	1.22:1	1.50:1	1.00:1	1.22:1	—

Table 2: Age Group Distribution Across Dengue Severity Groups

Age Group	DWWS	DWS	Severe	Total	%
1 – 5 years	7 (35%)	5 (25%)	7 (35%)	19	31.7%
6 – 10 years	7 (35%)	9 (45%)	10 (50%)	26	43.3%
11 – 14 years	6 (30%)	6 (30%)	3 (15%)	15	25.0%
Total	**20 (100%)**	**20 (100%)**	**20 (100%)**	**60**	**100%**

Table 3: NS1 Antigen and IgM Dengue Positivity by Group

Serological Test	DWWS	DWS	Severe	Total (N=60)
NS1 Antigen Positive, n (%)	13 (65%)	15 (75%)	12 (60%)	40 (66.7%)
NS1 Antigen Negative, n (%)	7 (35%)	5 (25%)	8 (40%)	20 (33.3%)
IgM Dengue Positive, n (%)	14 (70%)	14 (70%)	14 (70%)	42 (70.0%)

IgM Dengue Negative, n (%)	6 (30%)	6 (30%)	6 (30%)	18 (30.0%)
Either Test Positive (Confirmed)	**20 (100%)**	**20 (100%)**	**20 (100%)**	**60 (100%)**

Table 4: Frequency of Clinical Symptoms Across Dengue Severity Groups

Clinical Feature	DWWS n=20 (%)	DWS n=20 (%)	Severe n=20 (%)	Chi-Square (χ2)	p-value	Significance
Fever	20 (100%)	20 (100%)	20 (100%)	—	—	—
Vomiting	5 (25%)	15 (75%)	16 (80%)	15.42	0.0004	Significant
Abdominal Pain	1 (5%)	12 (60%)	18 (90%)	29.77	<0.0001	Significant
Headache	7 (35%)	10 (50%)	13 (65%)	3.60	0.165	Not Significant
Arthralgia / Myalgia	4 (20%)	6 (30%)	8 (40%)	2.18	0.337	Not Significant
Bleeding Manifestations	1 (5%)	7 (35%)	14 (70%)	18.23	0.0001	Significant
Edema	0 (0%)	3 (15%)	10 (50%)	15.52	0.0004	Significant
Rash	0 (0%)	3 (15%)	6 (30%)	7.06	0.029	Significant
Petechiae	3 (15%)	6 (30%)	9 (45%)	4.29	0.117	Not Significant
Icterus	2 (10%)	4 (20%)	6 (30%)	2.73	0.256	Not Significant

Table 5: Organomegaly and Lymphadenopathy Across Severity Groups

Clinical Sign	DWWS	DWS	Severe	p-value
Hepatomegaly, n (%)	2 (10%)	9 (45%)	17 (85%)	<0.0001
Splenomegaly, n (%)	1 (5%)	2 (10%)	6 (30%)	0.064
Hepatosplenomegaly, n (%)	0 (0%)	2 (10%)	5 (25%)	0.018
Lymphadenopathy, n (%)	0 (0%)	0 (0%)	2 (10%)	0.127
Any Organomegaly, n (%)	**2 (10%)**	**9 (45%)**	**18 (90%)**	**<0.0001**

Table 6: Platelet Count across Dengue Severity Groups

Parameter	DWWS (G1)	DWS (G2)	Severe (G3)	F-value	p-value
Mean ± SD (×103/μL)	234.60±72.59	100.90±34.69	42.70±22.56	83.20	<0.0001
Median (×103/μL)	227.50	96.00	38.50	—	—
Range (×103/μL)	131 – 386	53 – 191	12 – 92	—	—
Thrombocytopenia (<100×103/μL), n (%)	1 (5%)	9 (45%)	20 (100%)	—	<0.0001
Post-hoc Pairwise Comparisons	G1 vs. G2: p<0.0001	G1 vs. G3: p<0.0001	G2 vs. G3: p<0.0001	—	—

Table 7: Total Leucocyte Count (TLC) Across Dengue Severity Groups

Parameter	DWWS (G1)	DWS (G2)	Severe (G3)	F-value	p-value
Mean ± SD (×103/μL)	5.51±1.34	5.27±1.58	4.24±1.46	4.26	0.019

Leucopenia (<5×103/μL), n (%)	5 (25%)	8 (40%)	14 (70%)	—	0.007
Post-hoc Pairwise Comparisons	G1 vs. G2: p=0.611	G1 vs. G3: p=0.007	G2 vs. G3: p=0.038	—	—

Table 8: Comparative Serum Lipid Profile Across Severity Groups

Lipid parameter (mg/dL)	**Statistic**	**DWWS (G1)**	**DWS (G2)**	**Severe (G3)**	**F-value**	**p-value**
Triglycerides	Mean ± SD	240.65 ± 34.95	191.85 ± 34.40	165.40 ± 24.18	29.24	<0.0001
	Median	245.0	192.5	168.0	—	—
	IQR	215.5–269.8	170.2–217.0	146.0–178.0	—	—
	Hypertriglyceridaemia (>150 mg/dL), n (%)	20 (100%)	17 (85%)	14 (70%)	—	0.023
	Post-hoc pairwise comparisons	G1 vs G2: p = 0.0001	G1 vs G3: p < 0.0001	G2 vs G3: p = 0.008	—	—
HDL cholesterol	Mean ± SD	44.10 ± 6.36	28.00 ± 5.51	19.35 ± 4.55	103.58	<0.0001
	Median	44.0	27.5	19.0	—	—
	Range	32–57	19–41	13–29	—	—
	Low HDL (<40 mg/dL), n (%)	5 (25%)	20 (100%)	20 (100%)	—	<0.0001
	Post-hoc pairwise comparisons	G1 vs G2: p < 0.0001	G1 vs G3: p < 0.0001	G2 vs G3: p < 0.0001	—	—
LDL cholesterol	Mean ± SD	86.50 ± 18.71	65.25 ± 12.81	47.75 ± 10.64	36.02	<0.0001
	Median	87.0	64.5	47.0	—	—
	Range	55–131	41–90	31–72	—	—
	Low LDL (<70 mg/dL), n (%)	5 (25%)	14 (70%)	20 (100%)	—	<0.0001
	Post-hoc pairwise comparisons	G1 vs G2: p = 0.0002	G1 vs G3: p < 0.0001	G2 vs G3: p < 0.0001	—	—
Total cholesterol	Mean ± SD	158.40 ± 19.66	127.10 ± 21.68	96.00 ± 13.03	56.91	<0.0001
	Median	157.5	126.5	96.0	—	—
	Range	121–196	87–171	74–121	—	—
	Hypocholesterolaemia (<140 mg/dL), n (%)	3 (15%)	13 (65%)	20 (100%)	—	<0.0001
	Post-hoc pairwise comparisons	G1 vs G2: p < 0.0001	G1 vs G3: p < 0.0001	G2 vs G3: p < 0.0001	—	—